DEVELOPMENT OF THE ADRENAL GLAND IN THE CROSSBRED GOAT

By

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THESIS

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COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that the thesis entitled "**DEVELOPMENT OF THE ADRENAL GLAND IN THE CROSSBRED GOAT**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled "**DEVELOPMENT OF THE ADRENAL GLAND IN THE CROSSBRED GOAT**" is a record of research work done independently by **Shri. N. Ashok**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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In fond memory of
my mother

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Introduction

INTRODUCTION

The adrenal glands were first described and termed as "glandulae Renibus incumbentes" by Eustacchio in the year 1563. For the first time a life maintaining function and important features of deficiency state of this gland were ascribed by Addison in 1855.

The adrenal is a composite gland consisting of a medulla surrounded by a cortex. The gland is under dual control - hormonal and nervous (Coupland, 1975). The cortex and the medulla have separate ontogenic origin, the former being a mesodermal derivative while the latter arises from neuroectoderm (Weiner, 1975). It is the cortex and not the medulla which is essential for maintaining life. The presence of functional adrenal glands are essential for the normal postnatal development of the enzymic activities in the liver (Sereni *et al.*, 1959).

The adrenal cortex elaborates three kinds of steroid hormones which regulate two vital functions in the body. The water metabolism and electrolyte balance are regulated by the mineralocorticoid hormone (aldosterone) secreted from the zona glomerulosa and the carbohydrate metabolism by the glucocorticoids (Cortisol, Corticosterone etc.) secreted from

the zona fasciculata of the adrenal cortex. The third type of hormone secreted from the zona reticularis performs anabolic and androgenic functions. The medullary hormones are amines with effects similar to those of post ganglionic sympathetic neurons (Dickson, 1984).

There are wide variations in the degree of development of adrenal medulla at birth among different species. The sympathoadrenal system maintains foetal homeostasis as well as contributes to the maturation of the foetal lung (Walters and Oliver, 1978). During birth, catecholamines released by the foetal adrenal medulla play a key role in the adaptation of the new born to the extrauterine life (Slotkin and Seidler, 1988). The adrenal cortex and the medulla must be considered as an integrated functional unit in both the prenatal and postnatal mammal (Boshier *et al.*, 1989).

No precise study concerning foetal development of the caprine adrenal gland has been seen reported so far. In view of the prime importance of the gland during pregnancy and its key role in the initiation of parturition in several mammalian species, studies on the adrenal development in the goat was considered appropriate. Hence the present study by morphological, histological, histochemical and histometric approaches, was therefore undertaken with the objectives:

a. to trace the development of the entire gland, its cortex, medulla and each cortical zone, and

b. to evaluate the significance of growth of the adrenal gland in relation to body weight and age of the foetuses till term and of the kids upto six months postpartum.

Review of Literature

REVIEW OF LITERATURE

2.1 GROSS FEATURES

Adrenals are located in the retroperitoneum. The size and shape of the adrenal varied between right and left glands and also with species. In cows, the right one is roughly 'V' shaped lying against the medial surface at the canial pole of the right kidney. The left one is roughly `C' shaped and located on the midline just caudal to the cranial mesenteric artery on the medial face of the caudal vena cava cranial to the left kidney. In sheep, both the glands are bean shaped with the left gland slightly longer than the right (Venzke, 1975).

The left gland was slightly heavier than the right in cattle and sheep (Thwaites and Edey, 1970; Venzke, 1975). Warriss (1981) reported that the left gland was approximately 10.9 per cent heavier than the right in pigs. However, Nagra *et al.* (1989) noticed no difference in the weight, length, breadth and thickness between the right and the left adrenal glands in buffaloes.

2.2 DEVELOPMENT

2.2.1 Morphogenesis

In most mammalian species the adrenals have been reported to assume their characteristic shapes in the early foetal life itself (Hagar, 1965).

In sheep foetuses, a sharp increase in the weight of adrenal glands just before birth was recorded by several authors (Comline and Silver, 1961; Thurley, 1972). Jost (1975) opined that during the first part of development, the adrenals grew more rapidly than the whole body in many species. Boshier *et al.* (1980) and Boshier and Holloway (1989) observed a spurt in the adrenal weight in lamb foetuses from 136 days to birth.

Tiniakos *et al.* (1979) found that the weight of adrenal glands increased with the body weight in pig foetuses, but the adrenal to body weight ratio decreased continuously after birth. Lohse and First (1981) recorded a faster increase in the adrenal weight than the foetal weight during the last week of gestation in pigs. It was also observed that the adrenal to body weight ratio increased greatly during last 10 days of gestation. In gilts, the weight of the adrenal glands increased with age (Evans *et al.*, 1988).

In horse, the gland showed a steady increase in weight from fourth month of pregnancy till term (Yamauchi, 1979), while in foetal sheep and pigs the weight increased abruptly near term (Comline and Silver, 1961; Thurley, 1972; Lohse and First, 1981). This was attributed to the growth of adrenal cortex rather than the medulla. In West African dwarf goats, Osuagwuh and Aire (1992) observed a significant relationship between the adrenal weight and the gestational age.

2.2.2 Histogenesis

2.2.2.1 Prenatal period

The cortex and the medulla of the mammalian adrenal gland are united together although they are unrelated developmentally, structurally and functionally (Idelman, 1970).

There are different views regarding the formation of the primordia of the adrenal gland. According to the most accepted one (Venzke, 1975; Upadhyay and Zamboni, 1982) the first sign was noticed in the coelomic mesothelial cells located anteromedial to the metanephros. There the cells underwent active proliferation and infiltrated into the subjacent mesenchyme where they condensed into a discrete mass - the cortical anlage or the primordium of the adrenal cortex. The neuroblasts migrated from the nearby sympathetic ganglia

penetrated the medial aspect of the cortical primordium and transformed into chromaffinoblasts or sympathochromaffin cells which became the medullary primordium in domestic animals (Banks, 1981; Bielanska-Osuchowska, 1989a, 1989c; Boshier and Holloway, 1989). These chromaffin cells were reported to migrate between the cortical cells and finally occupied the centre of the gland as the medulla.

Uotila (1940) and Lanman (1953) noted that the foetal cortical cells first appeared in three weeks, while the adult type cortical cells in six weeks old human foetus. The occurrence of this zone in man and monkeys was reported by several authors (Lanman, 1957; Benirchke and Richart, 1964; Jost, 1975).

Soffer *et al.* (1961) reported that the true cortex in man continued to grow and develop as the age advanced while the foetal cortex degenerated rapidly after birth. The chromaffin cell differentiation occurred at a later stage and was completed at birth. However, according to Eberlein (1971) the invasion of sympathocytoblasts began at about 44 days of foetal life.

Lever (1955) reported that the primordium appeared in 12-13 days old rat embryo and the zonation was established only after 17 days of gestation. Invasion of the cortical

rudiment by sympathochromaffin cells started by 16th day and continued upto the late prenatal period.

According to Idelman (1970) the differentiation of zona glomerulosa started by 18 days and it was completed by 20 days in rat foetus. The gland was separated from the coelomic epithelium at 15 days. El-Maghraby and Lever (1980) noticed adrenal anlage at 16 days of intrauterine life in rats.

Ehrlich *et al.* (1989) found that the chromaffin cells originated from the neural crest and migrated to the emerging adrenal gland in rat foetuses. Seidl and Unsicker (1991) observed the commencement of chromaffin cell differentiation between 16 and 17 days of gestation in rats.

In bovine foetus, the cortex formation started between 28 and 33 days while the differentiation of a marginal zone and penetration of nerve fibres at about 50 days of intrauterine life. At four months, two types of cells were recognized in the medulla (Hager, 1965).

Alexander *et al.* (1968) reported that the histological appearance of the adrenal glands of sheep foetuses differed from that of lambs and ewes. In foetus, the cells of zona glomerulosa were arranged in irregular clusters and the cells of zona fasciculata in sheets without any pattern. The zona reticularis was not differentiated. The cells of the zona

glomerulosa were larger than those of the zona fasciculata in foetus while they were smaller in lambs and ewes.

In sheep, Thurley (1972) noted a steady growth of medulla throughout the foetal period with a sharp increase in the area of cortex just before birth.

Robinson *et al.* (1979) identified the adrenal anlage in 30 day old sheep foetuses. Between 35 and 60 days of age future cortical cells and a few future medullary cells arranged in small groups were observed. Capsule became apparent by 40 days of age. Histological differentiation of the gland started from 40 to 60 days of age with radial arrangement of cellular cords and formation of delicate connective tissue and capillaries in the parenchyma. After 60 days of age, the cortex showed two zones and the chromaffin cells became concentrated at the centre.

Boshier *et al.* (1980) reported that in 136 day old sheep foetuses, though the adrenocortical zonation was present, the three zones were not clearly demarcated. A stroma carrying blood vessels passed radially into the medulla. In full term foetuses, the three zones were well defined.

Pronounced growth of adrenal cortex was observed near birth in foetal sheep and goat between 136 and 144 days due to

hypertrophy and hyperplasia of the cells (Durand *et al.*, 1978; Liggins *et al.*, 1979; Hakeem *et al.*, 1993).

Upadhyay and Zamboni (1982) detected adrenal cortex primordium in 28 day old sheep foetuses. Till 31 days, the adrenal maintained anatomical continuity with the involuting giant glomerulus of the mesonephros. They concluded that the mesonephros was the primary source of the adrenal cortex.

Boshier and Holloway (1989) observed two periods of rapid growth separated by a period of reduced growth in the adrenal development of foetal sheep. The first period extended upto midgestation, then slowed to 120 days and thereafter the second growth period began.

According to Boshier *et al.* (1989), by day 53, in sheep foetuses whorls and columns of migratory sympathochromaffin cells containing both phaeochromoblasts and phaeochromocytes reached the cortical primordium and were seen among the cortical cells. By 100 days of development, corticomedullary separation was completed.

Nicolle and Bosc (1990) observed hypertrophy and hyperplasia of the cortical cells, particularly in the zona fasciculata, after 132 days and of the medullary cells after 144 days of pregnancy in sheep foetuses. A direct influence

of foetal pituitary on the cortical growth particularly on the zona fasciculata was also established.

Hakeem *et al.* (1993) recorded thin fibroelastic capsule and trabeculae extending into the zona glomerulosa in goats early in the foetal life, eventhough the reticular fibres appeared only in the later part of prenatal development. However, the cortical zonation was not clear until the late foetal life. The medulla was larger than the cortex during foetal life and the medullary cells were irregular and randomly scattered. Differentiation of the epinephrine and the norepinephrine cells took place before birth.

In guinea pigs, zonation of the adrenal was apparent by midgestation. By 27 days, the capsule was distinct and a sub capsular zone was apparent. The cells of this zone were later arranged in arched cords of zona glomerulosa. The inner zone of larger cells arranged in irregular cords, subsequently differentiated into zona fasciculata and zona reticularis by 50 to 55 days of gestation. Chromaffin cells began to migrate into the gland from its medial side by 24 days of foetal life (Black, 1972).

Albano *et al.* (1976) observed a clear differentiation and beginning of zonation of the adrenal cortex and a complete migration of the medullary cells to the centre of the gland by 24th day of development in foetal rabbits.

According to Yamauchi (1979) the adrenal medulla was thicker than the cortex in equine foetuses. The cortex with glomerular and fascicular zones was established by 4th month of pregnancy, however, the reticular zone developed only late in the foetal life. Hypertrophy of the cells of the zona fasciculata was also recorded during the last third of gestation.

In foetal pig, the process of adrenocortical zonation began by day 89 and was completed by day 113 of gestation (Lohse and First, 1981). Contrary to this Gutte *et al.* (1986) reported that the development of glomerular and fascicular zones in pigs started by day 80 of foetal life while the reticular zone developed only after birth.

Bielanska-Osuchowska (1989a; 1989b) observed the primordia of the adrenal cortex in pig embryos as early as 21 days of gestation near the mesonephros. Differentiation started by about 27th day of pregnancy. Large groups of chromaffinoblasts first penetrated the primordium. Between 31st and 35th days of pregnancy, signs of capsule and cortex development were evident. In the subcapsular region of the foetal adrenal, undifferentiated and differentiating cells were recorded.

2.2.2.2 Postnatal period

Kangarloo *et al.* (1986) studied postnatal changes in the adrenals of man and observed that with increasing age, the cortex became smaller and the medulla relatively larger. After one year of age the gland appeared similar to that of adult.

The general structure of the adrenal cortex in the postnatal sheep was similar to that in older foetuses. The zona reticularis was well differentiated with a distinct pattern and cellular structure. When compared to the older foetuses, in postnatal animals the cortex was much thicker (Alexander *et al.*, 1968).

Tischler et al. (1989) opined that both epinephrine and norepinephrine cells in the adrenal medulla of rats proliferated throughout life.

According to Pignatelli *et al.* (1995), at birth the medullary cells were seen as clusters instead of a well defined central zone, in rats. The staining affinity of the inner cortical zone progressively increased upto day 20 postpartum which paralleled the hormone secretion from the cortex.

Hullinger (1978) reported that in dogs, the prominent zona arcuata developed only after birth. By about three months all the cortical zones including the zona intermedia were well defined. The newly formed zona arcuata displaced the remaining cellular layer to an inward position where it formed the zona intermedia.

Tiniakos *et al.* (1979) noticed that in pigs, the zona fasciculata developed at a faster rate than the zona glomerulosa during the postnatal period. Moreover, there was no sign of involution of the cortex either before or after birth.

2.3 HISTOMORPHOLOGY

2.3.1 Stroma

The adrenal gland in domestic animals possessed a dense connective tissue capsule from which numerous septa extended into the parenchyma. The capsule contained a large number of parenchymal cells called capsular blastema, the superficial cells of which were small while the deeper ones were large (Trautmann and Fiebiger, 1957; Prasad and Yadava, 1974; Dellmann and Brown, 1981; Nagra *et al.*, 1989; Hakeem *et al.*, 1993).

Fahmy *et al.* (1965) and Al-Bagadadi (1969) noticed that the trabeculae radiating from the capsule anastomosed below the zona glomerulosa and formed an incomplete subglomerular connective tissue layer in camels. They also described an outer fibrous and an inner cellular parts for the capsule.

Prasad and Yadava (1972) and Ashok *et al.* (1994a) noticed collagen, elastic, reticular and smooth muscle fibres in the adrenal capsule of Indian buffaloes. Similar observations were made in goats (Hakeem *et al.*, 1993) and in other domestic animals also (Dellmann, 1993). It was reported that the corticomedullary junction was delineated mainly by reticular fibres. Collagen together with reticular fibres surrounded the groups of cells in the zona glomerulosa while they were arranged in radial columns in the zona fasciculata and in irregular fashion in the zona reticularis.

Medullary stroma was also made of the same group of fibres, but arranged in an alveolar fashion. Elastic fibres were scattered in the medulla and also in association with larger blood vessels. However, Prasad and Sinha (1981a) recorded only a very few elastic fibres in the adrenal medulla of the bullock and the horse.

Hinson *et al.* (1989) recorded mast cells in the walls of the arterioles in rats. They suggested that these mast cells modulate both vascular and secretory responses in the intact

adrenal gland. Jamdar and Ema (1982a) and Hakeem *et al.* (1993) reported the presence of melanocytes in the adrenal capsule of goats.

In buffaloes, the cellular population of the capsule included fibroblasts, melanin pigment cells and a few mast cells in addition to the clusters of undifferentiated cortical cells. Islands of well developed zona glomerulosa cells were also recorded (Ashok *et al.*, 1994a).

2.3.2 Parenchyma

The parenchyma of the adrenal gland was divided into an outer cortex and an inner medulla in all animals. The cortex was further subdivided into a zona glomerulosa, zona fasciculata and zona reticularis (Fahmy *et al.*, 1965; Al-Bagadadi, 1969; Prasad and Yadava, 1974; Venzke, 1975; Ganguli and Ahsan, 1978; Lohse and First, 1981; Banks, 1981; Nagra *et al.*, 1989; Dellmann, 1993).

The division of the mammalian adrenal cortex into three concentric zones was first made by Arnold (1866) and he coined the terms zona glomerulosa, zona fasciculata and zona reticularis. Gottschau (1883) described the structure and arrangement of parenchymal elements of these zones. In lipid-poor adrenals of the cow, sheep, goat and the horse, the

zonation between the zona fasciculata and the reticularis was less distinct (Long, 1975).

In addition to the three classic zones, a sudanophobic zone known as zona intermedia, has been described in several domestic and experimental animals (Cater and Lever, 1954; Ito, 1959; Bloodworth and Powers, 1968; Dickson, 1984; Dellmann, 1993; Ashok *et al.*, 1994a).

Demarcation between the cortex and the medulla was abrupt and distinct in squirrel monkeys (Penney and Brown, 1971) and in buffaloes (Prasad and Yadava, 1974). However, Ganguli and Ahsan (1978) noticed irregular line of demarcation between the two parts in goats. According to Banks (1981), the corticomedullary junction was sharply delineated or highly interdigitated in all domestic animals.

Otsuka (1962) recorded cortical tissue in the adrenal medulla of goats. Prasad and Sinha (1981a) recorded patches of cortical tissue in the adrenal medulla of horse and goat. Similarly, existence of medullary tissue in the cortex have been reported in sheep (Prasad and Sinha, 1984), in donkeys (Jamdar and Ema, 1982b; Abdalla and Ali, 1988-89), in buffaloes (Ashok *et al.*, 1994a) and in rats (Pignatelli *et al.*, 1995).

There are different theories of adrenocortical zonation. The `cell migration' or `escalator theory' (Gottschau, 1883) and its modification (Mitchell, 1948) were disproved by Walker and Rennels (1961) and Hunt and Hunt (1964; 1966).

The `theory of functional zonation' (Swann, 1940) proposed that the zona glomerulosa was relatively independant of pituitary control as against the other two zones. This notion of functional zonation provided the best working hypothesis to explain the structural zonation of the cortex.

Tonutti (1951) and Chester Jones (1957) suggested `the transformation field theory' according to which the zona fasciculata was the actively secreting part of the gland, while the other two zones were areas of reserve cells that could be transformed into actively secreting cells upon stimulation by ACTH.

2.3.2.1 Cortex

According to Soffer *et al.* (1961) cortical cells arose, functioned and perished off in one zone itself. They also reported cortical regeneration from the capsule.

In mammals, cells of zona glomerulosa were arranged in loops or whorls giving an acinar appearance. In the zona fasciculata, the cells were arranged in columns while in the

zona reticularis they were arranged at random. In the cow and sheep, the zona fasciculata was divided into inner and outer portions (Dickson, 1984).

2.3.2.1.1 Zona glomerulosa

Ganguli and Ahsan (1978) and Hakeem *et al.* (1993) recorded whorl like arrangement of cuboidal cells with basophilic, vacuolated cytoplasm and centrally placed nucleus in the zona glomerulosa of goats.

Prasad and Sinha (1984) reported that the zona glomerulosa was not very prominent in goats, sheep and pigs. They noticed a few medullary cells trapped within this zone.

In horses, carnivores and pigs, the zona glomerulosa consisted of curved cords or arcades of cells, while in ruminants and man they occurred in clusters (Venzke, 1975). In man, carnivores, horses and pigs the cells were columnar while in other species they were polyhedral. In man and ruminants, basophilic granules were also reported in the cells of zona glomerulosa (Banks, 1981). In buffaloes, the polyhedral cells of the zona glomerulosa had prominent oval or spherical nuclei and were arranged in clusters (Ashok *et al.*, 1994a).

2.3.2.1.2 Zona fasciculata

The middle cortical zone, zona fasciculata was the widest in all mammals and consisted of cuboidal or polyhedral cells arranged in radial cords separated by sinusoids (Idelman, 1970; Venzke, 1975; Banks, 1981).

The cells of the outer two-thirds of the zone were larger and possessed large, vesicular nuclei and a foamy cytoplasm. They were referred to as spongiocytes. Cells of the inner one-third were more basophilic and contained less lipid in domestic animals (Venzke, 1975; Dellmann, 1993).

Belloni *et al.* (1987) reported the presence of lipofuscin pigments in the cytoplasm of the cells of the inner portion of the zone in man.

2.3.2.1.3 Zona reticularis

This innermost zone was comprised of freely anastomosing irregular network of cords of cuboidal or polyhedral cells. The cells resembled those of zona fasciculata, but the lipid content was less. The cytoplasm was darker and the nuclei were heterochromatic and pyknotic (Soffer *et al.*, 1961; Long and Jones, 1967; Banks, 1981; Belloni *et al.*, 1987; Magalhaes *et al.*, 1988; Dellmann, 1993).

Idelman (1970) reported lysosomes and lipofuscin pigments in the zona fasciculata and the zona reticularis in several mammalian species with an abundance in the latter zone.

In goats Ganguli and Ahsan (1978) recorded two types of cells viz. dark basophilic and light basophilic cells towards the inner and outer parts of the zone respectively.

2.3.2.2 Medulla

The primary constituents of the adrenal medulla included glandular cells, ganglion cells, venules and capillaries. The columnar or polygonal glandular cells possessed large, vesicular nuclei and were polarised ie. one pole opposed to a capillary and the other to a venule. Their cytoplasm contained fine, granular, chromaffin positive granules and hence were referred to as chromaffin cells or pheochrome cells. Among the glandular cells, ganglion cells were scattered randomly (Banks, 1981). The chromaffin cells are concerned with the elaboration of epinephrine and norepinephrine (Soffer *et al.*, 1961).

By a special staining technique, Wood (1963) detected two types of glandular cells in the adrenal medulla of various experimental animals. Similar types of cells were identified in buffaloes (Prasad and Yadava, 1973; 1974; Nagra *et al.*, 1989; Ashok *et al.*, 1994a) and goats (Prasad and Sinha,

1981a). The epinephrine cells possessed eccenteric nuclei and brownish to purple granular cytoplasm as against the centrally located nuclei and yellow granular cytoplasm of the norepinephrine cells.

In the pig, Katsnelson (1964) observed that the epinephrine cells were picrinophilic while the norepinephrine cells were fuchsinophilic. Well defined boundary, central nucleus, finely granular cytoplasm and prismatic shape were the characteristics of the epinephrine cells. The cells formed cords around the wide venous sinuses. Norepinephrine cells were spherical or polygonal in shape and had indistinct boundary. The cytoplasm was coarsely granulated and the cells were arranged in spherical agglomerates around small capillaries. Similar findings were recorded in the adrenal medulla of several species of animals by Smollich (1966). Bloodworth and Powers (1968) reported similar observation in the adrenal medulla of dog. Ashok *et al.* (1994a) reported that the epinephrine cells were larger and darker than norepinephrine cells in buffaloes.

There existed a specific pattern of distribution of epinephrine and norepinephrine cells within the adrenal medulla. In domestic animals (Iskander and Mikhail, 1966; Prasad and Yadava, 1973; Ashok *et al.*, 1994a) including goats (Prasad and Sinha, 1981a), the epinephrine cells were arranged

in the outer zone and the norepinephrine cells in the inner zone of the medulla. Eventhen, the occurrence of one cell type in the territory of another type was also common.

In the camel, Fahmy *et al.* (1965) noticed that the ganglion cells were few and the chromaffin cells were arranged in oval groups. Unlike in other ruminants, in camels the norepinephrine cells were found in groups scattered among the epinephrine cells (Abdalla and Ali, 1988-89).

Smollich (1965; 1966) reported extensive degeneration of medullary epithelium and follicular formations resembling thyroid follicles in the peripheral zone of the medulla in ruminants.

2.4 HISTOCHEMISTRY

2.4.1 Carbohydrates

PAS positive granules were recorded in the cells of zona glomerulosa in bovines (Yamauchi, 1961) and in the cortical cells located in the medulla of goats (Otsuka, 1962).

In buffaloes (Prasad and Yadava, 1974; Nagra *et al.*, 1989) and in various other domestic animals (Prasad and Sinha, 1981b), a strong PAS positive reaction was recorded in the capsule, trabeculae and the cells of zona reticularis. The

reaction was moderate in the zona fasciculata and in the medulla, while no reaction was observed in the cells of zona glomerulosa.

Bielanska-Osuchowska (1989a) noticed PAS positive material between the cells of foetal cortex and the capillary walls of the developing adrenal cortex in the pig.

Planel and Guilhem (1956) demonstrated glycogen granules in the zona glomerulosa in man. However Long and Jones (1967) recorded glycogen in the reticularis also. In the rat embryo, glycogen granules were noticed in proximity to the liposomes and believed that they were concerned with the elaboration and regeneration of lipid resources of the gland. Albano *et al.* (1976) reported that in the rabbit foetus, from day 16 onwards, the future cortical cells showed large accumulation of glycogen.

No reaction was detected for acid mucopolysaccharides in the zona glomerulosa of bullocks, while a weak positive reaction was seen in other domestic animals (Prasad and Sinha, 1981b). In buffaloes, only the capsule, trabeculae and the medullary cells revealed moderate reaction for acid mucopolysaccharides, while no reaction was detected in the cortical cells (Ashok *et al.*, 1994b).

2.4.2 Enzymes

2.4.2.1 Alkaline phosphatase

Alkaline phosphatase activity varied with species and sex of animals. Dempsey *et al.* (1949) observed that in male rats the activity gradually increased from glomerulosa to reticularis zone as against the female rats which had no activity in the fasciculata and reticularis. In the goat enzyme activity was strong in the zona fasciculata and reticularis but was weak in the zona glomerulosa. In sheep, the zona glomerulosa was negative as against in bovines, in which the activity was absent in the zona reticularis (Nicander, 1952; Yoffey, 1955).

According to Aso *et al.* (1980), the alkaline phosphatase activity was limited to zona reticularis alone in man, while Soffer *et al.* (1961) recorded intense activity in the zona reticularis, moderate in the zona fasciculata and little or no activity in the zona glomerulosa.

In rabbits, sheep, pigs and goats, the reaction was more intense in the zona fasciculata and zona reticularis and less in the zona glomerulosa. In the horse and cattle it was higher in glomerulosa and less in reticularis (Arvy, 1971). However, Bhattacharya and Saigal (1985) and Nanda *et al.* (1993) observed a downward gradation of alkaline phosphatase

activity in the adrenal cortex of goats, being most prominent in zona glomerulosa followed by fasciculata and reticularis.

According to Bielanska-Osuchowska (1989a, 1989b), the adrenal cortical cells of pig embryos showed alkaline phosphatase activity and the number of such cells increased with development. The activity was weak in the undifferentiated subcapsular cells, while it was totally absent in the chromaffinoblasts which migrated into the cortical primordia.

2.4.2.2 Acid phosphatase

The acid phosphatase activity was uniform throughout the cortex in cats, rabbits and squirrel monkeys, while the activity was stronger in the zona glomerulosa in horses and bovines (Nicander, 1952; Penney and Brown, 1971). Aso *et al.* (1980) observed enzyme activity mainly in the cells of zona reticularis, in man. Moreover, within the cells the activity was localised in the lysosomes.

According to Bielanska-Osuchowska (1989a), in the adrenal cortex of foetal pig, only a few cells situated directly under the capsule showed mild activity of acid phosphatase. However, in older foetuses, a weak activity was noticed throughout the cortex.

In buffaloes, the activity was moderate in the zona glomerulosa and zona fasciculata and weak in the zona reticularis. The capsule and trabeculae did not show any activity. The activity was stronger in the medulla than the cortex (Ashok *et al.*, 1994b).

Soffer *et al.* (1961) reported that only epinephrine cells contained acid phosphatase while norepinephrine cells lacked this enzyme in man.

2.4.3 Catecholamines

Miller (1926) observed that in the developing adrenal gland of mouse the catecholamines were present in the cells of medullary anlage by 15th day of intrauterine life at which time the medullary cells just began to penetrate between the cortical cells.

The time of appearance of positive chromaffin reaction in medullary precursor cells varied between the different species of animals (El-Maghraby and Lever, 1980).

Wood (1963) reported dark iodate positive islets of the norepinephrine secreting cells scattered throughout the medulla in rats. In rabbits, the norepinephrine content of the medulla was reported to be practically nil.

According to Weiner (1975), the catecholamine content of the adrenal gland of the rat, guinea pig, rabbit, cattle and man during the foetal life was exclusively norepinephrine. Epinephrine began to appear in the postnatal period when the adrenal cortical development was most active.

In the adrenal medulla of buffaloes, only the norepinephrine cells reacted to potassium iodate (Ashok *et al.*, 1994b).

2.4.4 Lipids

Deane (1958) and Dickson (1984) were of the opinion that the lipid inclusions in the adrenals were the sites of storage of cholesterol esters, the important precursors of steroid hormones. Decreased content of histochemically detectable lipid would be an indication of increased functional activity. However, Nussdorfer *et al.* (1978) and Nussdorfer (1980) suggested that chronic stimulation of the cortical cells resulted in an increased lipid content after an initial decrease while prolonged depression caused a decrease in lipid content after a transient increase, both in the zona glomerulosa and the zona fasciculata.

The concentration of lipid material in the adrenal cortical cells was high in carnivores, man and rats. It was maximum in the zona fasciculata in most mammalian species

(Soffer *et al.*, 1961). According to Belloni *et al.* (1987) the cells of zona glomerulosa contained sparse lipid droplets while the zona fasciculata and reticularis contained lipid laden cells, in man.

The cortical cells surrounding the central vein as well as the cells of the cortical islands within the medullary region of the adrenal in goats contained lipid droplets (Otsuka, 1962; Ganguli and Ahsan, 1978).

Among the cortical zones, maximum lipid concentration was observed in the zona glomerulosa in merino ewes (Thwaites and Edey, 1970). According to Idelman (1970) in the dog this zone contained only a few lipid droplets.

In the adrenal gland of various domestic animals including goats, lipid content was maximum in the zona fasciculata while the other two cortical zones contained only a moderate quantity (Prasad and Sinha, 1981b). According to Ashok *et al.* (1994b), the lipid concentration was minimum in the zona glomerulosa and moderate in the other two zones in buffaloes.

In guinea pigs, by 27 days of gestation, when the adrenal blastema got differentiated into a narrow outer zone and a thicker inner zone, abundant lipid droplets were recorded in the inner zone. Between 30 and 50 days lipid droplets became

larger and more numerous in the outer part of the inner zone, but decreased in the inner part of the inner zone (Black, 1972).

Albano *et al.* (1976) reported that in the adrenal gland of rabbit foetuses, lipid droplets began to accumulate towards the end of gestation.

Robinson *et al.* (1979) noticed occasional lipid droplets within the cell mass of the adrenal cortical primordium between 30 and 60 days of gestation in sheep foetuses. After the differentiation of the inner and outer zones at 60 to 120 days, the cells of the outer zone were characterised by small lipid droplets.

According to Gutte *et al.* (1986) lipids were present in the adrenal cortex of foetal pigs from 80th day of development.

2.5 MICROMETRY

The percentage contribution of zona glomerulosa, zona fasciculata and zona reticularis to the total volume of the cortex was 27, 50 and 23 per cent respectively in dogs (Hullinger, 1978); 18, 53 and 29 per cent in camels (Abdalla and Ali, 1988-89) and 15, 70 and 15 per cent in goats (Hakeem *et al.*, 1993).

The cortex to medulla ratio declined from 1.0 to 0.4 with increasing foetal age in equines (Yamauchi, 1979).

Boshier *et al.* (1980) observed that during the last two weeks of gestation in foetal sheep, the thickness of cortex increased four times. However, the zona glomerulosa slightly decreased in size. The cortical growth rate was linear and the medullary growth was negligible at this period.

In foetal pigs, significant increase in the volume of adrenal cortex occurred between 89 and 97 days and 105 and 113 days. (Lohse and First, 1981). In sheep foetuses, Durand *et al.* (1978) reported the greatest increase in the size of cortex towards the end of gestation.

Nicolle and Bosc (1990) also noticed increase in the volume of the cortex and the medulla in sheep foetuses near term.

According to Hakeem *et al.* (1993) thickness of the capsule, cortex and the medulla increased with age of the foetuses in goats. In postnatal animals, the thickness of the capsule, zona fasciculata and the medulla increased while that of the zona glomerulosa and reticularis decreased from first month till ninth month.

Material and Methods

MATERIALS AND METHODS

Development of adrenal glands of goat was studied during prenatal and postnatal periods using subjects of various age groups.

1. Prenatal group (Group I)

Prenatal study was conducted on 55 foetuses comprising of 17 sexually indifferent and 38 male foetuses collected from the municipal slaughter house, Trichur (Table 1). Immediately after collection, body weight and crown-rump length (CRL) were recorded. Then the age of the foetuses was calculated from the formula,

$W^{1/3} = 0.096\ (t-30)$, derived by Singh *et al.* (1979) for goat foetuses

where,

W = Body weight of the foetus in g

t = Age of the foetus in days

Based on the age, the foetuses were divided into six subgroups as shown in Table 2.

For histological and histochemical investigations, embryos upto 43 days were fixed in toto. From embryos of 44

days to 58 days, either dorsal region with mesonephros and metanephros or the whole trunk (ie., thorax and abdomen) was resected and fixed. From still larger foetuses of 59 days to 152 days of age, both the right and left adrenal glands were removed after recording the gross relations. However, a few larger foetuses upto 75 days of age, were fixed in toto and serial sections were taken to trace the migratory pattern of medullary precursor cells to the cortical primordium.

The glands were weighed separately and the length, breadth and thickness were recorded at the point where they measured maximum. In subjects of 40 to 58 days, length and breadth of the glands were measured using an ocular micrometer, since the organ was not harvestable. Thereafter the measurements were taken by using a Vernier Callipers.

2. Postnatal group (Group II)

The materials were collected from 45 apparently healthy, male goats of known ages from the Kerala Agricultural University Goat Farm, Mannuthy (Table 3), and the animals were grouped as shown in Table 4.

After sacrificing the animals, the abdomen was opened. The position, relations and shape of both the adrenals were recorded. The right and the left glands were weighed separately and length, breadth and thickness of both the

glands were taken at the point where they measured maximum. Each gland was cut across into small pieces and fixed in different fixatives. Depending on the requirements the following fixatives were used:

1. 10 per cent neutral buffered formalin
2. Helly's fluid
3. Bouin's fluid
4. Chilled Acetone (4°C)
5. 90 per cent alcohol
6. 10 per cent potassium iodate (Drury and Wallington, 1980)
7. Formol-Dichromate (pH 4.0-4.2) (Wood, 1963)

Frozen sections of 20 μm thickness were also taken for the lipid studies.

After fixation in the appropriate fixatives, the materials were processed for paraffin embedding. Tissues for the histological techniques were processed in high melting paraffin (MP-58-60°C) and sections of 4-5 μm thickness were made. For histochemical studies tissues were processed in low melting paraffin (MP-40-42°C) and 6 μm thick sections were cut.

Serial sections of 6 μm thickness were taken from smaller embryos for locating the primordia and studying the adrenal glands at early stages of development.

The following histological staining techniques were employed on paraffin sections.

1. Ehrlich's haematoxylin and eosin staining technique for routine histological studies (Luna, 1968).

2. Mallory's Phosphotungstic acid haematoxylin (PTAH) method for collagen fibres (Luna, 1968).

3. Van Gieson's method for collagen (Luna, 1968)

4. Gomoris Aldehyde fuchsin technique for elastic fibres (Drury and Wallington, 1980)

5. Gridley's method for reticular fibres (Gridley, 1951)

6. Masson's trichrome method for connective tissue and muscle fibres (Luna, 1968)

The different cytological staining techniques employed were the following:

1. Hirano-Zimmerman method for nerve cells and fibres (Luna, 1968)

2. Unna's method for mast cells (Luna, 1968)

3. Fontana-Masson's silver method for pigment cells and argentaffin granules (Luna, 1968)

4. AFIP method for lipofuscin pigments (Luna, 1968)

5. Wood's technique for medullary catecholamine cells (Wood, 1963)

For the histochemical studies, the following methods were employed:

1. Periodic acid Schiff's (PAS) reaction for carbohydrates (Bancroft and Stevens, 1977).

2. Alcian blue method (pH 2.5) for acid mucopolysaccharides (Luna, 1968)

3. Best's Carmine method for glycogen (Bancroft and Stevens, 1977).

4. Modified Gomori's method for alkaline phosphatase (Pearse, 1977)

5. Naphthol AS-B1 phosphate method for acid phosphatase (Barka, 1960)

6. Oil Red `O' in propylene glycol method for lipids (Luna, 1968)

7. Iodate method for noradrenaline (Drury and Wallington, 1980)

The data on the following physical parameters were analysed (Snedecor and Cochran, 1967) to find out the significance, if any:

1. Age on the combined weight of adrenal glands

2. Body weight on the combined weight of adrenal glands.

3. Age on the percentage contribution of combined adrenal weight to body weight.

4. Age on the weight of right and left adrenal glands.

5. Age on the length, breadth and thickness of right and left adrenals.

Correlation of the above parameters and the percentage contribution of right and left glands to the combined adrenal weight were also studied.

Micrometry

To measure changes in width of the cortex and the medulla during gestation and postnatal period, the width of entire cortex, different cortical zones and diameter of the medulla were measured using an ocular micrometer. Since the cortex and the medulla could not be differentiated in young foetuses, separate measurements of both the regions were recorded in

foetuses of 74 days and above. The zona glomerulosa was measured only after 78 days. Since the reticular zone could not be clearly demarcated till late pregnancy (ie., till 129 days of gestation), the thickness of the combined fascicular and reticular zones was measured at all stages in foetuses. Each gland was cut across at the centre and the cortical measurements were recorded at four different points and the average was taken. The diameter of the medulla was measured at two points and its average was taken.

Micrometry was recorded and the data were analysed (Snedecor and Cochran, 1967) for the following to find the significance, if any:

1. Age on the thickness of capsule.

2. Age on the width of entire cortex.

3. Age on the ratio of zona glomerulosa to zona fasciculata in kids, and zona glomerulosa to the rest of the cortex in foetuses.

4. Age on the diameter of medulla.

5. Age on the ratio of the cortex to medulla.

Correlation of the above parameters and the proportion of different zones of the adrenal cortex to the entire cortex were also studied.

Table 1. Crown-rump length (CRL), body weight and the age of foetuses used

Sl.No.	CRL (mm)	Body weight (g)	Age of the foetus (in days)
1.	8	0.180	17
2.	9	0.190	18
3.	12	0.220	21
4.	14	0.318	24
5.	16	0.484	27
6.	16	0.485	27
7.	17	0.670	30
8.	18	0.700	32
9.	18	0.720	32
10.	19	0.740	33
11.	21	0.800*	36
12.	22	0.940	40
13.	24	1.550	42
14.	24	1.600	42
15.	25	1.960	43
16.	28	2.600	44
17.	28	2.560	44
18.	54	4.450	47
19.	58	7.600	50
20.	57	7.500	50
21.	64	8.200	51
22.	80	15.500	56
23.	84	18.200	57
24.	84	19.000	58
25.	86	21.100	59
26.	88	22.400	59
27.	85	22.000	59
28.	86	21.500	59
29.	88	30.000	62

Sl.No.	CRL (mm)	Body weight (g)	Age of the foetus (in days)
30.	89	30.000	62
31.	92	35.000	64
32.	104	53.000	69
33.	103	56.000	70
34.	109	58.000	70
05.	120	70.000	73
36.	125	75.000	74
37.	128	80.000	75
38.	138	100.000	78
39.	150	140.000	84
40.	148	140.000	84
41.	190	240.000	95
42.	215	280.000	98
43.	220	320.000	101
44.	225	360.000	104
45.	235	400.000	107
46.	230	400.000	107
47.	245	540.000	115
48.	295	680.000	122
49.	287	680.000	122
50.	313	860.000	129
51.	320	860.000	129
52.	352	1100.000	138
53.	370	1220.000	141
54.	405	1700.000	147
55.	430	1800.000	152

* Below this, age is calculated from CRL

Table 2. CRL, body weight, age and number of foetuses in the prenatal group

Sub-groups	CRL range (mm)	Body weight range (g)	Age in days (range)	Number of foetuses
i	8-17	0.18-0.67	15-30	9
ii	18-28	0.70-2.56	31-45	8
iii	54-128	4.45-80.0	46-75	20
iv	138-225	100.00-360.00	76-105	7
v	235-320	400.00-860.00	106-135	7
vi	352-430	1100.00-1800.00	136-term	4
			Total	55

Table 3. Age and body weight of the animals used

Sl.No.	Age (in days)	Body weight (kg)
1.	Day old	1.95
2.	Day old	1.95
3.	Day old	2.35
4.	Day old	2.05
5.	Day old	2.14
6.	15	3.65
7.	15	3.80
8.	15	2.40
9.	15	2.80
10.	15	3.10
11.	30	4.50
12.	30	5.60
13.	30	4.10
14.	30	4.40
15.	30	5.20
16.	45	4.75
17.	45	8.00
18.	45	4.50
19.	45	4.60
20.	45	4.70
21.	60	8.40
22.	60	6.75
23.	60	4.75
24.	60	7.00
25.	60	6.85
26.	90	5.50
27.	90	7.20
28.	90	7.50
29.	90	7.30
30.	90	7.25

Sl.No.	Age (in days)	Body weight (kg)
31.	120	9.00
32.	120	10.50
33.	120	9.80
34.	120	9.60
35.	120	9.25
36.	150	13.00
37.	150	15.50
38.	150	14.00
39.	150	13.50
40.	150	14.50
41.	180	17.00
42.	180	17.50
43.	180	14.00
44.	180	18.50
45.	180	17.50

Table 4. Age, body weight and number of animals in the postnatal group

Sub-groups	Age in days	Body weight range (kg)	Number of animals
i	Day old	1.95-2.35	5
ii	15	2.40-3.80	5
iii	30	4.10-5.60	5
iv	45	4.50-8.00	5
v	60	4.75-8.40	5
vi	90	5.50-7.50	5
vii	120	9.00-10.50	5
viii	150	13.00-15.50	5
ix	180	14.00-18.50	5
		Total	45

Results

RESULTS

4.1 GROSS OBSERVATIONS

4.1.1 Shape

The right adrenal was roughly triangular and the left one elongated (Fig.1). Both the glands were very small and showed the characteristic shapes in early foetal life itself. They acquired a harvestable size around 59 days of gestation.

4.1.2 Weight

Weight of the adrenal gland at various stages are presented in Tables 6 and 7. The left and the right glands increased in weight from 59 days to full term. A spurt in the combined adrenal weight was noticed after 141 days of gestation (Fig.2). The adrenal weight was positively correlated with the age (r=0.883; Fig.3) and the body weight (r=0.979; Fig.4) of the foetuses. Weight of the right and the left adrenal glands as well as their combined weight was significantly higher after 106 days of gestation (Tables 5 and 6). The proportionate combined adrenal weight to body weight decreased after 59 days of gestation (Table 6). A negative correlation existed between the age and the percentage of combined adrenal gland weight to the body weight (r=-0.699).

After birth, the combined adrenal weight showed an increasing trend upto 180 days. However a marginal decrease at 30 days and a significant decrease at 45 days were noticed (Fig.5). A similar trend was noticed when the weight of the right and left glands studied separately (Tables 5 and 7). The combined weight of the adrenals was highly correlated with the age (r=0.927; Fig.5) as well as with the body weight (r=0.919; Fig.6). The proportionate combined weight of the adrenal glands to the body weight showed a decreasing trend during postnatal period. The decrease was significant at 30, 45 and 150 days eventhough a marginal increase was recorded at 90 and 120 days (Table 7). This parameter was negatively correlated with the age (r=-0.665).

Between prenatal and postnatal groups, the proportionate combined adrenal weight to body weight was highly significant, with a lower value for the postnatal group. Similarly, the weight of right and left glands as well as their combined weight were significantly higher in the postnatal group than that of the prenatal group (Table 8).

In the prenatal group the left adrenal was 4.6 per cent heavier than the right (Fig.7) while in the postnatal group, it was 5.4 per cent heavier (Fig.8). However, the difference in weight between the right and the left adrenals was not significant in both the groups studied (Table 9).

4.1.3 Size

Length, breadth and thickness of both the adrenals are presented in Tables 6 and 7.

Length of both the adrenals showed an increasing trend with the advancement of foetal age (Figs.9 and 10). The increase was significant between the subgroups studied (Tables 5 and 6). Length of right and left adrenals were positively correlated with foetal age (r=0.969 and r=0.904 respectively). The left adrenal was significantly longer than the right (Tables 6 and 9).

In the postnatal groups also, length of the adrenals showed an increasing trend. For the right gland this increase was significant in subgroups of 15, 90 and 150 days. However, a marginal decrease at 45 days and 120 days was also observed (Table 7; Fig.11). For the left gland a significant increase at 15, 60, 120 and 180 days and a significant decrease at 30 days postpartum was recorded (Table 7; Fig.12). Length of the right and the left glands were positively correlated with age (r=0.722 and r=0.809 respectively). The left gland was significantly longer than the right in the postnatal group also (Tables 7 and 9).

In the prenatal group, average breadth of the right and the left glands increased with age (Table 6; Figs.9 and 10).

There was a high positive correlation between foetal age and breadth of the right (r=0.901) and the left (r=0.920) adrenals.

In the postnatal group also, breadth of both the adrenals showed an increasing trend. However, a marginal decrease was noted at 45 days of age (Table 7; Figs.11 and 12). A positive correlation existed between the age and breadth of right (r=0.758) and left (r=0.586) adrenals.

Though the right gland was broader than the left in both prenatal and the postnatal groups, only in the latter it was significant (Table 9).

Thickness of the adrenals increased with foetal age (Figs.9 and 10). This increase was positively correlated for the right (r=0.936) and the left (r=0.819) glands.

In the postnatal group also the same trend was noticed (Figs.11 and 12), eventhough a marginal decrease was noted at 45 days. Thickness of the right and the left glands was positively correlated with age (r=0.770 and r=0.698 respectively).

In both the prenatal and the postnatal groups, right gland appeared to be thicker than the left but, a significant difference was noticed only in the postnatal group (Table 9).

4.1.4 Relations

In the early stages of development, adrenal primordium was related laterally to the mesonephros and ventro-laterally to the developing gonad. Both these structures had direct anatomical continuity with the developing adrenals. The glands were retroperitoneal in position.

The right adrenal was located on the cranio-medial aspect of the right kidney (Fig.13). The medial surface was somewhat flattened while the lateral moderately convex. The right crus of the diaphragm was related medially and the caudate process of the right lobe of the liver laterally. Ventro-medially the gland was related to the caudal vena cava. The base was in apposition with the cranial pole of the right kidney and the apex in the angle between the caudal vena cava and the liver.

The left adrenal was located approximately on the mid line, slightly anterior to the cranial pole of left kidney. It was lying little anterior to the level of the right adrenal (Fig.13). Laterally it was related to the dorsal sac of rumen, medially to the abdominal aorta.

4.2 MICROSCOPIC OBSERVATIONS

4.2.1 Histomorphology

4.2.1.1 Prenatal group

4.2.1.1.1 15-30 days of development

Adrenal primordia could not be detected in any of the foetuses under this group.

4.2.1.1.2 31-45 days of development

Adrenal anlage was first identified in 33 days old embryos. The area of adrenal development was characterised by whorls of cells anterior to the developing metanephros (Fig.14). These whorls of mesenchymal cells were seen on the ventrolateral aspect of dorsal aorta, along the dorsomedial part of the mesonephros. The primordium on the left side was located more cranially compared to that on the right side.

By about 36 days, these cells gradually began to organize into cords and dense aggregations. These cells possessed deeply eosinophilic cytoplasm with oval or spherical nuclei.

At 42 days, a very thin capsule composed of collagen fibres was visible surrounding the primordium. Fine collagen fibrils were noticed among the parenchymal cells also at this stage. The cells had indistinct borders and were more regularly organized into small groups and cords. Cellular

cords were separated by irregular spaces. The first sign of formation of a central vein was evident at this stage (Fig.15). Numerous mitotic figures were also recorded. The primordia at this stage was continuous with the mesonephros, developing metanephros and the gonad.

4.2.1.1.3 46-75 days of development

Here the glands increased in size and were related to the cranio-medial aspect of the metanephros. The continuity with the mesonephros and the gonad was not visible after 50 days.

In subjects of 50 days of development, capsule was well defined and the gland could be clearly demarcated from the surrounding structures. The parenchyma was arranged as irregular cellular cords. Migratory cells made their presence for the first time at this stage both within and around the cortical primordium as aggregations or solitary cells (Fig.16). These cells were smaller than the cortical cells and possessed dark, oval nuclei (Fig.17). Few cells seen within the parenchyma showed mild chromaffin reaction even at this stage (Fig.18).

At 58 days of development, the parenchymal cells just beneath the capsule started to form small clusters. The capsule was better developed and reticular fibres were first detected mainly in the capsule at this stage (Fig.19). The

migration of future medullary cells in whorls continued towards the cortical mass. The developing medullary cells in the parenchyma showed more intense chromaffin reaction.

By 62 days, the cords and clusters of cells beneath the capsule were more densely packed. The cellular cords towards the central part of the gland were loosely arranged. The capsule became thicker and the central vein was more prominent. The migratory cells started concentrating towards the central part of the gland and they showed the characteristic chromaffin reaction. Differentiation of these cells within the gland started with the appearance of two types of cells viz. the one with typical characters of multipolar neurons and the other with the features of glandular cells (Fig.20). The latter type had vesicular nucleus and chromaffin positive granules in their cytoplasm and were arranged in clusters.

At this stage, near the periphery of the gland, admixture of cortical and medullary cells were seen, while towards the centre, medullary cells were predominent.

The adrenal gland at 70 days of foetal age showed a very well developed capsule containing numerous capillaries, neurons and fibroblasts. Division of the capsule into an outer more fibrous and an inner more cellular layers was apparent. The cords and clusters of cells were separated by

sinusoids. The cortical cells possessed homogenous eosinophilic cytoplasm and were arranged in tightly packed cords and clusters. The centrally placed vesicular nucleus was darker compared to the medullary cells and showed peripheral condensation of chromatin. Nucleoli were prominent in most of the cells. The corticomedullary separation had started. Though the cortical and the medullary cells were intermingled with each other, the latter tended to concentrate towards the centre (Fig.21). The intensity of migration of the predetermined neural crest cells towards the developing gland diminished with foetal age. No migration was noticed after 70 days of foetal development.

Trabeculae were seen extending from the capsule into the parenchyma by 74 days. From this age onwards stray elastic fibres were also recorded in the capsule in addition to collagen and reticular fibres. The cells beneath the capsule were arranged as clusters (Fig.22). Concentration of the medullary cells towards the centre of the gland continued. Admixture of cortical and medullary cells were seen in the central part (Fig.23). Throughout the parenchyma sinusoids containing blood elements were observed. Cells with neuronal characters were also distributed throughout the gland.

4.2.1.1.4 76-105 days of development

A well defined capsule and trabeculae were present at 78 days. Chromaffin cells were concentrated more at the central region (Fig.24).

By 95 days of development, though the zona glomerulosa was distinguishable, the differentiation of zona fasciculata and zona reticularis had not yet started. Though the central region of the gland contained predominantly ~~the~~ medullary type of cells, a few cortical cells were also seen scattered. The cortex and the medulla were clearly demarcated at this age.

At 98 days subcapsular region was relatively narrow and almost free of chromaffin cells. Central part of the gland showed extensive distribution of chromaffin cells (Fig.25) with a few cortical cells either in groups or as solitary ones. Arrangement of cells into parallel cords indicated the initiation of zona fasciculata differentiation. The zona reticularis was yet to develop.

Stray smooth muscle fibres were noticed in the capsule in addition to the collagen, elastic and reticular fibres in foetuses of 101 days of age (Fig.26).

By 104 days of development, the radially arranged cellular cords of the zona fasciculata were better organized. The medulla at this stage contained two types of cells.

Larger cells with indistinct boundaries were mostly seen at the periphery of the medulla. Smaller cells with distinct cellular boundaries arranged mostly in groups or less frequently as solitary ones were seen throughout the medulla. The larger cells were few in number and possessed light nuclei while the smaller ones were numerous and had dark nuclei. A few cortical cells were also noticed in between the medullary cells as well as surrounding the central vein.

4.2.1.1.5 106-135 days of development

Highly vascularised capsule with numerous trabeculae was clearly divisible into an outer more fibrous and an inner more cellular layers (Fig.27). The zona glomerulosa was very well differentiated from the rest of the cortex. Organization of zona fasciculata continued, however differentiation of zona reticularis had not yet started. The groups of medullary cells were surrounded by delicate network of reticular fibres (Fig.28). Corticomedullary junction presented interdigitations. Within the medulla large number of cortical cells were noticed around the central vein.

Not much changes occurred in the structure of the gland till 122 days of foetal age.

By 122 days, thin strands of connective tissue comprising of collagen and reticular fibres were seen in between the

clusters of zona glomerulosa and around individual cells of the inner cortical zone. Cords of cells arranged in parallel and irregular arrays were seen beneath the zona glomerulosa representing the future zona fasciculata. Still deeper, irregular cellular cords, indicative of zona reticularis formation was also noticed (Fig.29).

By 129 days of development, the cortex was clearly divisible into a subcapsular zone, the zona glomerulosa containing clusters of cells; a zona fasciculata made up of parallel cell cords and an innermost zona reticularis comprising of irregular cell cords (Fig.30).

4.2.1.1.6 136-150 days of development

After 129 days of gestation, distinct zonation of the cortex and a medulla resembling that of the postnatal animals were present. The three classical zones of the cortex viz. zona glomerulosa, zona fasciculata and zona reticularis were evident. In the medulla, larger cells were loosely arranged in cords towards the periphery, while densely packed smaller cells occurred in clusters towards the central part. However, the former type was few in number. Medulla also contained the cortical cells surrounding the central vein (Fig.31).

The cortical cells had spherical, vesicular nuclei with prominent nucleoli. The cytoplasm was eosinophilic and finely

granular. The cells of zona glomerulosa were seen in clusters and were large with indistinct cell boundaries (Fig.32).

By the end of gestation, follicles of varying sizes, surrounded by firbous tissue were recorded in the medulla. These follicles contained a colloid material (Fig.33). Numerous irregular trabeculae were recorded in the medulla. The capsule of the gland at this stage contained a few flat, irregular cells with melanin pigment granules in the cytoplasm.

The width of the cortex particularly of the zona fasciculata increased steadily towards the end of gestation which was characterised by hypertrophy and hyperplasia of the cells.

4.2.1.2 Postnatal group

During postnatal period the histological appearance and the general structural plan of the adrenal gland did not differ much. However micrometry and the histochemistry of the gland showed some differences.

The gland was covered by a connective tissue capsule composed of collagen, elastic and reticular fibres. A few smooth muscle cells were also seen. From the capsule numerous trabeculae invaded the parenchyma to varying depths, some

of which even reached upto the medulla. The capsule and trabeculae contained numerous blood vessels.

The cellular components of the capsule included fibroblasts, occasional multipolar neurons and numerous melanocytes. Melanocytes were flat, elongated and branched with pigment granules in the cytoplasm (Fig.34). Capsule also contained a few ganglia of varying sizes made up of multiploar neurons and glial cells (Fig.35).

In the cellular part of the capsule groups of undifferentiated cells with pale, vesicular nuclei were recorded. The capsule also contained fully differentiated cortical cells resembling those of zona glomerulosa. These cells were arranged as groups or clusters within the capsule (Fig.36).

From the capsule and trabeculae, thin strands of connective tissue fibres invaded the parenchyma and formed the supporting framework of the gland. These strands surrounded each group of cells of the zona glomerulosa and the radially arranged cellular cords of the zona fasciculata. From the connective tissue strands of the zona fasciculata, thin fibres extended and surrounded individual cells of the zone. In the zona reticularis also, a similar connective tissue framework was observed.

The corticomedullary junction showed interdigitations of varying degrees (Fig.37). In the medulla, the stroma was disposed in an irregular fashion around groups and cords of cells (Fig.38). In some cases, the trabeculae from the capsule traversed the whole cortex and medulla and reached the central vein. The central vein possessed wide lumen with thin wall. Lining endothelium was supported by collagen and reticular fibres.

The parenchyma was broadly divisible into an outer cortex and an inner medulla. The cortex was further subdivided into zona glomerulosa, zona fasciculata and zona reticularis respectively from the periphery to the medulla. Mast cells were recorded occasionally in all the three cortical zones (Fig.39).

Medullary cells were seen trapped in all the cortical zones and also as extensions or podia into the cortex from the medulla. In a few cases these medullary podia extended even upto the capsule after passing through the cortex (Fig.40). Cortical cells also showed their presence in the medulla frequently, especially around the central vein. Usually these cells were seen along the trabeculae which indicate their path of migration.

A tightly packed zona intermedia was noticed in between the zona glomerulosa and the zona fasciculata (Fig.41). This

zone was found to be sudanophobic. During early postnatal life this zone was not visible. It could be clearly defined after 120 days of age.

Cells of the zona glomerulosa were polyhedral and arranged in irregular clusters and cords. Occasionally these cells migrated into the zona fasciculata along the trabeculae. The cells of zona glomerulosa had indistinct cellular boundaries. However, glomerulosa cells located in the capsule had distinct cell borders. The cytoplasm contained occasional vacuoles and basophilic granules. The vesicular nuclei showed peripheral condensation of chromatin and possessed distinct nucleoli. Nuclei were darker compared to those of fasciculata cells. The acidophilia of cytoplasm intensified gradually from the outer to the inner zone of the cortex (Fig.42).

Middle zone, the zona fasciculata was the widest and consisted of irregular, polyhedral or cuboidal cells arranged in radial cords of one or two cells thick (Fig.43). These cells were smaller than the cells of zona glomerulosa. The adjacent cellular cords were separated by sinusoids. The acidophilic cytoplasm in the cells of the outer portion of the zone was foamy or vacuolated thus the cells were identified as spongiocytes (Fig.44). Cytoplasmic vacuolation was less in the cells of the inner portion of the zone. The nuclei were larger and more vesicular than those of the other two cortical zones.

Innermost zone, the zona reticularis consisted of irregular or polyhedral cells arranged in anastomosing cords without any definite pattern (Fig.45). These cells were smaller with eosinophilic cytoplasm which contained tiny vacuoles and lipofuscin pigments (Fig.46).

The adrenal medulla could be divided into two zones with specific histologic characteristics. The peripheral zone consisted of larger cells with indistinct boundaries and were deeply eosinophilic compared to the cells of the central zone. These cells were arranged in cords and clusters and possessed eccentrically located vesicular nuclei (Fig.47).

The cells of the central zone were smaller and possessed clear cytoplasm, distinct cell boundaries and centrally placed nuclei. These cells were arranged in clusters or islets (Fig.48).

Large cells were identified as epinephrine secreting cells while the small ones were of norepinephrine secreting type. When stained by Wood's technique (Wood, 1963) epinephrine secreting cells showed homogenous, brownish to purple granular cytoplasm. The norepinephrine cells possessed less homogenous, yellow granular cytoplasm (Fig.49).

Less frequently small cells were seen towards the peripheral part of the medulla either singly or in small

groups. However, large cells were seldom seen in the central zone of the medulla.

The epinephrine cells predominated in number over the norepinehrine cells in postnatal animals. Number of epinephrine cells gradually increased from the day of birth till 180 days postpartum.

In addition to the glandular cells, the medulla contained large number of neurons. These cells had cytoplasmic processes and large, spherical or ovoid nuclei with prominent nucleoli.

At various stages of development the medulla showed follicles containing a homogenous, eosinophilic colloid like substance.

4.2.2 Histochemistry

4.2.2.1 Carbohydrates

4.2.2.1.1 Mucopolysaccharides and acid mucopolysaccharides

The cytoplasm of the cortical primordial cells in foetuses of 42 days showed moderate PAS positive reaction (Fig.50). More intense reaction was noticed in older foetuses. In foetuses of 75 days, the cluster of cells beneath the capsule representing the primitive zona

glomerulosa as well as the differentiating medullary cells showed a weak reaction while more centrally placed cords of cells representing the rest of the cortex had a strong reaction for PAS. The capsule of the adrenal also showed a positive reaction which intensified as age advanced. In full term foetuses, the zona glomerulosa and medulla showed a weak PAS reaction while zona fasciculata and zona reticularis were strongly positive.

In postnatal life, capsule and trabeculae showed strong PAS reaction. All three cortical zones were PAS positive. The reaction was weak in the zona glomerulosa and zona intermedia, moderate in the zona fasciculata and strong in the zona reticularis (Fig.51). In the medullary cells it was moderate, however the colloid and the stroma revealed a strong positive reaction.

The reaction for acid mucopolysaccharides in 73 days old foetuses was moderate in the capsule and weak in the cortical cells (Fig.52). However earlier to this no positive reaction for acid mucopolysaccharides was noticed. As the foetal age advanced, acid mucopolysaccharide content also increased in the cortical cells and showed a strong reaction after 122 days of foetal age. Medullary cells showed a moderate reaction.

During postnatal life, acid mucopolysaccharide concentration did not vary much at different ages in the

various regions of the gland. Moderate reaction was recorded in the capsule and the outer zona fasciculata. A more intense reaction was observed in the zona glomerulosa, inner part of zona fasciculata and zona reticularis (Fig.53). In the medulla, connective tissue reacted moderately while medullary cells revealed a weak reaction (Fig.54).

4.2.2.1.2 Glycogen

Glycogen was detected in both the primitive cortical and medullary cells of young foetuses (Fig.55). The reaction was stronger in the subcapsular zone. With the advancement of pregnancy the intensity of the reaction slightly decreased in the cortical cells eventhough the medullary cells showed a moderate reaction throughout the foetal period.

In all the animals studied, glycogen content was more in the cells of zona glomerulosa and zona reticularis than those of the zona fasciculata and the medulla.

4.2.2.2 Enzymes

4.2.2.2.1 Alkaline phosphatase

In foetuses of 50 days, the adrenal cortical cells presented alkaline phosphatase activity. A strong activity was noticed upto 84 days of foetal life. Thereafter the activity remained moderate upto 122 days and then increased

till term. The activity was weak in the cluster of cells situated just beneath the capsule which increased towards the central part of the cortex (Fig.56). The medullary cells lacked the enzyme activity. Towards the terminal stage of gestation, alkaline phosphatase activity was weak in the zona glomerulosa, moderate in the zona fasciculata and the medulla and strong in the zona reticularis.

During postnatal life, there was a decreasing trend in the enzyme activity from the zona glomerulosa to the zona reticularis.

The medulla revealed a moderate activity. Among the different age groups studied a strong activity was recorded in the zona glomerulosa upto 30 days of age. At 45 days, a decrease in the enzyme activity was noticed in this zone. At 60 days, the activity increased once again and remained strong upto 180 days (Table 10).

In the zona fasciculata also a similar trend was noticed. From 45 to 60 days the activity increased and was moderate upto 180 days.

In the zona reticularis, the activity was very strong upto 15 days which gradually decreased from 30 to 45 days postpartum. Thereafter a weak reaction was recorded upto 180 days.

4.2.2.2.2 Acid Phosphatase

Moderate activity of acid phosphatase was recorded in the cortical cells just below the capsule in foetuses from 58 days to term. Medullary cells showed strong enzyme activity throughout foetal life. In other cortical cells, the enzyme activity was very weak or even absent in foetuses upto 122 days of age. Thereafter a moderate activity was recorded in these cells also till term.

During postnatal life, capsule and trabeculae were negative for acid phosphatase; however the endothelia of blood vessels revealed a moderate activity.

Among the cortical zones, zona glomerulosa and zona fasciculata showed stronger activity than zona reticularis till 45 days of age. Thereafter upto 180 days the activity was weak in the zona glomerulosa and moderate in the other two zones.

Within the zona fasciculata, enzyme activity was more intense in the inner fasciculata than the outer fasciculata cells. Cells of the zona reticularis revealed a moderate activity (Fig.57).

When compared to the cortex, the medullary cells had a stronger acid phosphatase activity (Fig.58). Within the

medulla itself the peripheral zone of cells reacted more strongly than the central zone of cells.

Among the animals of different age groups studied, both the zona glomerulosa and the zona fasciculata revealed a stronger enzymic activity upto 30 days postpartum. But the reaction was weak in these zones at 45 days. From 45 days to 180 days the activity was weak in the zona glomerulosa and moderate in the zona fasciculata (Table 10).

In the zona reticularis, the activity was moderate throughout the postnatal period.

4.2.2.3 Catecholamines

Medullary cells till 98 days of foetal life were exclusively of norepinephrine type. These cells were first detected at 50 days of foetal life. The chromaffin positive cells were also recorded first at this stage. These cells reacted positively to potassium iodate and occurred singly or in groups. They were distributed throughout the parenchyma. As the age advanced, they increased in number and the response to iodate reaction in the central medullary zone intensified. A few epinephrine cells could be detected in 98 days foetal adrenal, but a substantial increase was seen in number from 129 days to term.

In the animals of postnatal group, the number of epinephrine cells increased greatly and exceeded the norepinephrine cells.

Dark, iodate positive islets of norepinephrine secreting cells were found throughout the medulla. They were more frequently encountered in the central part near the central vein. (Fig.59). The intensity of iodate reaction was not affected by age in the postnatal animals.

4.2.2.2 Lipids (Table 11)

Fine lipid droplets were detected in the primitive cortical cells from 50 days of foetal life onwards. Generally the lipid droplets were smaller in the foetal adrenal. There was a gradual increase in the lipid content in the cortical cells with the advancement of foetal age. The primitive medullary cells were free of lipid.

Till 74 days of foetal age, lipid droplets were uniformly distributed in the cortical cells. However, after this age it concentrated more in the clusters of cells in the subcapsular region which represented the primitive zona glomerulosa. Cells in the rest of the cortex showed a moderate to low lipid content.

From 84 to 122 days of foetal age, the cortical cells other than the cells of primitive zona glomerulosa contained

only very little quantity of lipid. The masses of cortical cells seen at the central part of the gland also contained a moderate quantity of lipid.

After 122 days, the total quantity of stainable lipid increased steadily in various cortical zones until term. After 141 days, the foetal adrenal cortex contained heavy accumulation of lipid.

The capsule and the medulla were free of lipid, while the islands of cortical cells in the capsule as well as in the medulla contained lipid in both the prenatal and the postnatal groups studied (Fig.60). Likewise, the zona intermedia found in the postnatal group was devoid of lipid.

Adrenal cortex of the day old kids contained heavy accumulation of lipid droplets (Fig.61). By day 15 postpartum, though the lipid content remained high in the zona fasciculata and the zona reticularis, a gradual depletion was recorded from the zona glomerulosa. By 30 days, there was a depletion from all the cortical zones particularly from the zona glomerulosa and the outer zona fasciculata. In the zona reticularis a moderate concentration of lipid remained.

By 45 days of age, again a very heavy accumulation was observed in all the cortical zones including the zona glomerulosa (Fig.62). The accumulation was maximum in the

zona fasciculata particularly in the outer fasciculata region. This was the stage at which maximum intensity of lipids was noticed in all the three cortical zones. After 45 days, the lipid gradually depleted from the cortex particularly from the zona fasciculata and zona reticularis and attained a moderate concentration by 60 days. However, the zona glomerulosa contained substantial amount of lipid (Fig.63).

The depletion of lipid from the zona glomerulosa continued upto 120 days of age. In the zona fasciculata and the zona reticularis the lipid concentration was moderate and remained without much change from 60 to 180 days. Between 120 and 180 days lipid content of zona glomerulosa was very little.

4.2.3 Micrometry

4.2.3.1 Thickness of the capsule

Tables 13 and 14 show the thickness of capsule at various stages. Thickness significantly increased from 51 days to term (Table 13; Fig.64). In animals of postnatal group also the capsular thickness showed an increasing trend (Fig.65). While comparing the prenatal and the postnatal groups the capsular thickness increased significantly in the postnatal group from the prenatal group (Table 15). Capsular thickness

was positively correlated with age in the prenatal (r=0.904) and the postnatal (r=0.771) groups.

4.2.3.2 Width of the cortical zones

Tables 13 and 14 show width of the various cortical zones.

Width of the zona glomerulosa increased significantly from 78 days to 135 days followed by a significant decrease towards the end of gestation (Fig.64). During postnatal period width of zona glomerulosa increased steadily from day old to 180 days of age with a marginal decrease at 45 days (Fig.65). Width of the zona glomerulosa increased significantly in the postnatal group compared to the prenatal group (Table 15). Width of the zona glomerulosa was positively correlated with age in the prenatal (r=0.360) and the postnatal (r=0.741) groups.

In the prenatal group, width of the cortex other than zona glomerulosa increased from 78 days to full term (Table 13; Fig.64). The width was positively correlated with foetal age (r=0.918).

In the postnatal group, width of the zona fasciculata increased from day of birth to 180 days of age. However there was a significant decrease at 45 days of age (Table 14;

Fig.65). A high positive correlation existed between the age and width of zona fasciculata (r=0.852).

After an initial significant increase in the width of zona reticularis in postnatal animals from day old to 15 days, there was a decreasing trend towards 180 days of age (Table 14; Fig.65). A negative correlation existed between age and width of zona reticularis (r=-0.814).

Width of the entire cortex recorded a significant increase from 74 days to term in the prenatal group (Table 13; Fig.64).

In the postnatal group, after a significant increase from the day of birth to 30 days, a significant decrease was observed at 45 days. Following this, there was a significant increase upto 180 days (Table 14; Fig.65). In the postnatal group, a significant increase was noticed in the total width of cortex compared to the prenatal group (Table 15).

Width of the cortex was positively correlated with age in both prenatal (r=0.932) and postnatal (r=0.809) groups.

4.2.3.3 Diameter of the medulla

The values are presented in Tables 13 and 14.

An increasing trend was observed in the width of the medulla both in prenatal (Fig.64) and postnatal groups

(Fig.65). In the prenatal group it increased steadily from 74 days of gestation to term (Table 13).

Diameter of the medulla was significantly higher in the postnatal group compared to the prenatal group (Table 15).

There was a positive correlation between age and width of medulla both in the prenatal (r=0.617) and the postnatal (r=0.935) groups.

4.2.3.4 Zona glomerulosa/rest of the cortex ratio

The values are given in Table 13. This ratio was calculated in the prenatal group only since the zona reticularis was not distinguishable till late in the foetal period. Though the ratio increased marginally from 78 days to 135 days, there was a significant decrease after this period till term (Fig.66). This ratio was negatively correlated with foetal age (r=-0.592).

4.2.3.5 Zona glomerulosa/zona fasciculata ratio

The values are presented in table 14.

This ratio was calculated for the postnatal group. Following an increasing trend from day old to 60 days with a marginal decrease at 30 days, the ratio decreased till 120

days. Thereafter a marginal increase was noticed at 150 and 180 days (Fig.67).

4.2.3.6 Cortex/medulla ratio

The values are presented in tables 13 and 14.

This ratio showed an increasing trend in the prenatal group from 74 days to full term (Fig.66). However, in the postnatal group, the ratio followed a decreasing trend from day old to 45 days of age. The decrease was significant at 30 and 45 days. Thereafter a marginal increase was noticed upto 180 days (Fig.67).

The cortex/medulla ratio was significantly higher in the postnatal group compared to the prenatal group (Table 15).

4.2.3.7 Cortical width and combined adrenal weight relationship

In the prenatal group, as the adrenal weight increased, width of the entire cortex also increased. This increase was attributed to the increased growth of cortex other than zona glomerulosa. However, zona glomerulosa showed a decreasing trend after an initial slight increase (Fig.68).

In the postnatal group also, width of the cortex increased as the adrenal weight increased. Here the increase

was mainly due to the growth of zona fasciculata. Zona glomerulosa also increased in width, eventhough it was marginal. However, the width of zona reticularis decreased as the weight of the adrenals increased (Fig.69).

4.2.3.8 Percentage contribution of different cortical zones

On an average, during the prenatal period, the zona glomerulosa contributed 22.91 per cent and the rest of the cortex 77.09 per cent to the total width of the cortex (Fig.70).

In the postnatal life, zona glomerulosa contributed 16.71 per cent, zona fasciculata 74.16 per cent and zona reticularis 9.13 per cent to the total cortical width (Fig.71).

Table 5. ANOVA table for the effect of age on physical parameters of the adrenals in prenatal and postnatal groups

Characters	Prenatal group			Postnatal group		
	Treatment d.f.	Error d.f.	MS	Treatment d.f.	Error d.f.	MS
Weight of right adrenal	3	25	0.014**	8	36	0.110**
Weight of left adrenal	3	25	0.016**	8	36	0.128**
Combined weight of right and left adrenals	3	25	0.060**	8	36	0.472**
Percentage contribution of combined adrenal weight to body weight	3	25	0.145**	8	36	0.056**
Length of right adrenal	4	34	0.621**	8	36	0.092**
Breadth of right adrenal	4	34	0.247**	8	36	0.035**
Thickness of right adrenal	3	25	0.050**	8	36	0.038**
Length of left adrenal	4	34	0.787**	8	36	0.288**
Breadth of left adrenal	4	34	0.198**	8	36	0.019**
Thickness of left adrenal	3	25	0.023**	8	36	0.012**

* - P≤0.05
** - P≤0.01

Table 6. Comparison of physical parameters of right and left adrenals in fœtuses (Mean±SE)

Characters	Subgroups				
	ii (n=6)	iii (n=11)	iv (n=7)	v (n=7)	vi (n=4)
Weight of right adrenal (g)	-	c 0.006± 0.01	c 0.018± 0.01	b 0.044± 0.01	a 0.121± 0.01
Weight of left adrenal (g)	-	c 0.007± 0.01	c 0.021± 0.01	b 0.049± 0.01	a 0.132± 0.01
Combined weight of right and left adrenals (g)	-	c 0.013± 0.01	c 0.039± 0.02	b 0.093± 0.02	a 0.253± 0.02
Combined adrenal weight in proportion to body weight (%)	-	a 0.030± 0.002	b 0.018± 0.001	b 0.014± 0.001	b 0.019± 0.002
Length of right adrenal (cm)	e 0.050± 0.05	# d 0.267± 0.03	c 0.536± 0.04	b 0.667± 0.04	a 0.865± 0.06
Length of left adrenal (cm)	d 0.083± 0.08	# c 0.402± 0.05	b 0.606± 0.07	b 0.761± 0.07	a 1.098± 0.09
Breadth of right adrenal (cm)	d 0.026± 0.03	# c 0.228± 0.02	b 0.399± 0.03	b 0.470± 0.03	a 0.517± 0.04
Breadth of left adrenal (cm)	d 0.041± 0.03	# c 0.218± 0.02	b 0.336± 0.03	a 0.426± 0.03	a 0.515± 0.04
Thickness of right adrenal (cm)	-	d 0.191± 0.01	c 0.263± 0.01	b 0.311± 0.01	a 0.402± 0.02
Thickness of left adrenal (cm)	-	b 0.190± 0.01	b 0.203± 0.01	a 0.283± 0.01	a 0.310± 0.02

n=15

Note: Means of a character with same superscripts are not significantly different ($P \leq 0.05$)

Table 7. Comparison of physical parameters of right and left adrenals in postnatal group (Mean±SE)

n=5

Characters	Subgroups								
	i	ii	iii	iv	v	vi	vii	viii	ix
Weight of right adrenal (g)	e 0.174± 0.02	cd 0.257± 0.02	cd 0.249± 0.02	de 0.212± 0.02	cd 0.240± 0.02	c 0.289± 0.02	b 0.477± 0.02	a 0.544± 0.02	a 0.551± 0.02
Weight of left adrenal (g)	f 0.179± 0.01	d 0.313± 0.01	d 0.286± 0.01	e 0.234± 0.01	d 0.290± 0.01	d 0.325± 0.01	c 0.501± 0.01	b 0.558± 0.01	a 0.650± 0.01
Combined weight of right and left adrenals (g)	g 0.353± 0.03	de 0.570± 0.03	de 0.535± 0.03	f 0.445± 0.03	e 0.530± 0.03	d 0.614± 0.03	c 0.978± 0.03	b 1.101± 0.03	a 1.201± 0.03
Combined weight in proportion to body weight (%)	a 0.017± 0.0008	a 0.019± 0.0018	b 0.011± 0.0011	cd 0.009± 0.001	cd 0.008± 0.0009	cd 0.009± 0.001	bc 0.010± 0.0004	d 0.008± 0.0009	d 0.007± 0.0004
Length of right adrenal (cm)	e 0.930± 0.05	cd 1.112± 0.05	bcd 1.146± 0.05	de 1.046± 0.05	cd 1.098± 0.05	ab 1.254± 0.05	bc 1.184± 0.05	a 1.320± 0.05	a 1.360± 0.05
Length of left adrenal (cm)	e 1.138± 0.04	bc 1.598± 0.04	d 1.426± 0.04	d 1.446± 0.04	c 1.564± 0.04	bc 1.616± 0.04	b 1.678± 0.04	bc 1.670± 0.04	a 2.032± 0.04
Breadth of right adrenal (cm)	c 0.734± 0.03	c 0.736± 0.03	c 0.758± 0.03	c 0.734± 0.03	c 0.768± 0.03	c 0.772± 0.03	c 0.788± 0.03	b 0.894± 0.03	a 0.974± 0.03
Breadth of left adrenal (cm)	cd 0.616± 0.03	bcd 0.648± 0.03	bcd 0.672± 0.03	cd 0.640± 0.03	cd 0.645± 0.03	cd 0.642± 0.03	bc 0.678± 0.03	ab 0.726± 0.03	a 0.798± 0.03
Thickness of right adrenal (cm)	b 0.440± 0.03	b 0.502± 0.03	b 0.478± 0.03	b 0.474± 0.03	b 0.480± 0.03	b 0.478± 0.03	a 0.630± 0.03	a 0.642± 0.03	a 0.664± 0.03
Thickness of left adrenal (cm)	d 0.394± 0.02	bc 0.454± 0.02	bc 0.472± 0.02	bc 0.460± 0.02	cd 0.444± 0.02	cd 0.438± 0.02	ab 0.504± 0.02	a 0.534± 0.02	a 0.552± 0.02

Note: Means of a character with same superscripts are not significantly different (P≤0.05)

Table 8. Comparison of adrenal weight between prenatal and postnatal groups

Characters	't' value
Weight of the right gland	12.876*
Weight of the left gland	13.512*
Combined weight of the glands	13.281*
Proportionate combined weight of the adernals to the body weight	6.143*

* Significant ($P \leq 0.05$)

Table 9. Comparison of physical parameters of right and left adrenals in prenatal and postnatal groups

Characters	't' value	
	Prenatal group	Postnatal group
Weight	0.289	1.201
Length	1.582*	9.574*
Breadth	0.539	6.847*
Thickness	1.867	3.520*

* Significant ($P \leq 0.05$)

Table 10. Phosphatase activity of the adrenal in postnatal group

Enzymes	Sub-groups	Capsule and trabeculae	Cortex			Medulla
			ZG	ZF	ZR	
Alkaline phosphatase	i	-	++++	++++	++++	++
	ii	-	++++	++++	++++	++
	iii	-	++++	++++	+++	++
	iv	-	++	+	+	++
	v	-	+++	++	+	++
	vi	-	+++	++	+	++
	vii	-	+++	++	+	++
	viii	-	+++	++	+	++
	ix	-	+++	++	+	++
Acid phosphatase	i	-	+++	+++	++	++++
	ii	-	+++	+++	++	++++
	iii	-	+++	+++	++	++++
	iv	-	++	++	++	++++
	v	-	+	++	++	++++
	vi	-	+	++	++	++++
	vii	-	+	++	++	++++
	viii	-	+	++	++	++++
	ix	-	+	++	++	++++

ZG : Zona glomerulosa
ZF : Zona fasciculata
ZR : Zona reticularis

\+ : Weak
++ : Moderate
+++ : Strong
++++ : Very strong
\- : Negative

Table 11. Lipid content of the adrenal in postnatal group

Sub-groups	Capsule and trabeculae	Cortex			Medulla
		ZG	ZF	ZR	
i	-	++	+++	+++	-
ii	-	+	+++	+++	-
iii	-	+	++	++	-
iv	-	+++	++++	++++	-
v	-	+++	++	++	-
vi	-	++	++	++	-
vii	-	+	++	++	-
viii	-	+	++	++	-
ix	-	+	++	++	-

ZG : Zona glomerulosa
ZF : Zona fasciculata
ZR : Zona reticularis

\- : Absent
\+ : Lightly concentrated
++ : Moderately concentrated
+++ : Heavily concentrated
++++ : Very heavily concentrated

Table 12. ANOVA table for the effect of age on micrometrical parameters of the adrenal in prenatal and postnatal groups

	Characters	Prenatal group			Postnatal group		
		Treatment d.f.	Error d.f.	MS	Treatment d.f.	Error d.f.	MS
	Thickness of capsule	3	27	2734.307**	8	36	3324.382**
	Width of ZG	2	21	11793.151**	8	36	2450.828**
+	Width of ZF+ZR	2	21	237724.571**	-	-	-
	Width of ZF	-	-	-	8	36	162628.897**
	Width of ZR	-	-	-	8	36	3004.618**
	Width of entire cortex	3	23	244122.045**	8	36	168048.502**
	Diameter of medulla	3	23	114094.392	8	36	1432302.305**
+	Ratio of ZG/ZF+ZR	2	21	0.104**	-	-	-
	Ratio of ZG/ZF	-	-	-	8	36	0.002**
	Ratio of cortex/medulla	3	23	0.096**	8	36	0.038**

** : P≤0.01
ZG : Zona glomerulosa
ZF : Zona fasciculata
ZR : Zona reticularis

+ These measurements were made for prenatal group only since the zona fasciculata and zona reticularis were indistinguishable till late in the foetal life. For the postnatal group zona fasciculata and zona reticularis were measured separately.

Table 13. Comparison of micrometrical parameters of the adrenal in prenatal group (Mean±S.E)

Characters	Subgroups			
	iii (n=3)	iv (n=6)	v (n=5)	vi (n=4)
Thickness of the capsule (μ)	# c 23.227± 1.93	b 48.628± 3.16	ab 57.300± 3.46	a 61.347± 3.86
Width of the ZG (μ)	-	c 68.940± 10.77	a 147.592± 8.34	b 109.565± 9.33
Width of the ZF±ZR (μ)	-	c 194.243± 45.12	b 343.807± 34.95	a 558.679± 39.08
Width of the entire cortex (μ)	d 240.980± 63.56	cd 263.183± 44.95	b 491.399± 34.82	a 668.244± 38.93
Diameter of the medulla (μ)	c 1108.520± 85.80	c 1166.628± 60.67	bc 1253.335± 46.99	a 1426.992± 52.54
Ratio of the ZG/ZF+ZR (μ)	-	a 0.370± 0.04	a 0.434± 0.03	b 0.220± 0.03
Ratio of the entire cortex/medulla	b 0.216± 0.06	b 0.226± 0.04	a 0.399± 0.03	a 0.474± 0.03

\# : n=16
ZG : Zona glomerulosa
ZF+ZR : Cortex excluding zona glomerulosa (ie. rest of the cortex)

Note : Means of a character with same superscripts are not significantly different ($P \leq 0.05$)

Table 14. Comparison of micrometrical parameters of the adrenals in postnatal group (Mean±SE)

n=5

Characters	Subgroups								
	i	ii	iii	iv	v	vi	vii	viii	ix
Thickness of capsule (μ)	d 65.052± 5.47	cd 80.052± 5.47	cd 75.728± 5.47	c 81.472± 5.47	c 82.390± 5.47	c 81.704± 5.47	c 81.624± 5.47	b 110.176± 5.47	a 150.970± 5.47
Width of ZG (μ)	d 107.464± 7.47	c 131.456± 7.47	c 133.960± 7.47	c 129.996± 7.47	c 140.582± 7.47	c 137.114± 7.47	bc 141.804± 7.47	b 162.902± 7.47	a 186.166± 7.47
Width of ZF (μ)	d 678.886± 27.43	d 679.646± 27.43	c 770.156± 27.43	d 680.524± 27.43	d 684.116± 27.43	cd 705.802± 27.43	b 947.982± 27.43	b 1018.652± 27.43	a 1132.276± 27.43
Width of ZR (μ)	b 130.376± 4.71	a 144.890± 4.71	b 123.986± 4.71	c 109.414± 4.71	cd 102.540± 4.71	cd 93.844± 4.71	de 81.590± 4.71	e 77.412± 4.71	e 79.246± 4.71
Width of entire cortex (μ)	de 916.726± 34.14	cd 988.402± 34.14	c 1056.574± 34.14	de 919.934± 34.14	de 942.688± 34.14	de 953.798± 34.14	b 1188.932± 34.14	b 1278.304± 34.14	a 1412.914± 34.14
Diameter of medulla (μ)	d 1420.400 70.49	d 1579.596± 70.49	c 1832.428± 70.49	bc 2002.254± 70.49	b 2121.376± 70.49	b 2190.400± 70.49	a 2782.926± 70.49	a 2751.592± 70.49	a 2892.924 70.49
Ratio of ZG/ZF	bcd 0.158± 0.01	abcd 0.187± 0.01	abcd 0.168± 0.01	ab 0.198± 0.01	a 0.200± 0.01	abc 0.191± 0.01	d 0.147± 0.01	cd 0.156± 0.01	abcd 0.162± 0.01
Ratio of cortex/ medulla	a 0.652± 0.02	ab 0.626± 0.02	b 0.585± 0.02	c 0.443± 0.02	c 0.445± 0.02	c 0.434± 0.02	c 0.441± 0.02	c 0.465± 0.02	c 0.488± 0.02

ZG : Zona glomerulosa
ZF : Zona fasciculata
ZR : Zona reticularis

Note: Means of a character with same superscripts are not significantly different (P≤0.05)

Table 15. Comparison of micrometrical parameters of the adrenals between prenatal and postnatal groups

Characters	`t' value
Thickness of capsule	9.937*
Width of zona glomerulosa	2.836*
Width of entire cortex	12.964*
Diameter of medulla	10.490*
Ratio of cortex/medulla	4.858*

* Significant ($P \leq 0.05$)

Fig.1 Right (R) and left (L) adrenals from a 60 days old kid showing their shapes

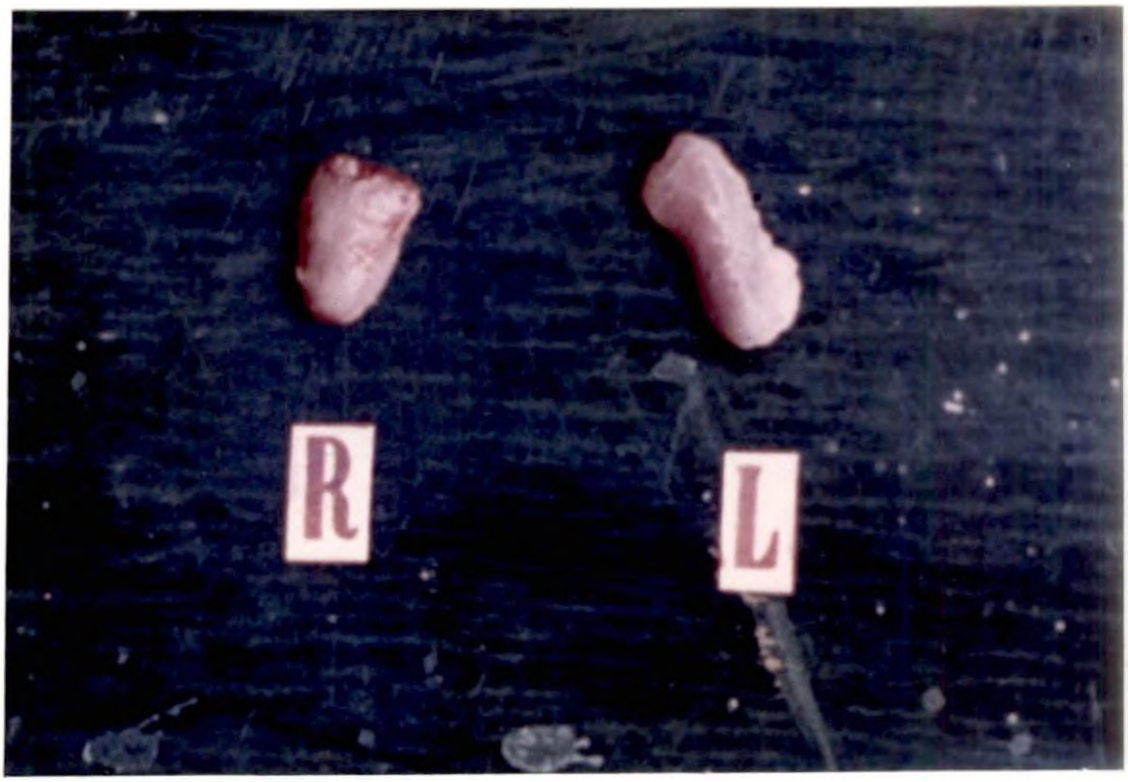

R
L

Fig. 2 Relationship between foetal age and combined weight of adrenals after 120 days of gestation

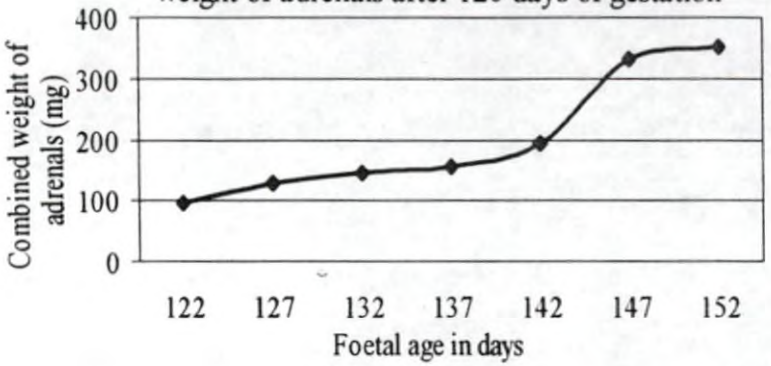


Fig. 3 Relationship between age and combined weight of adrenals in prenatal group

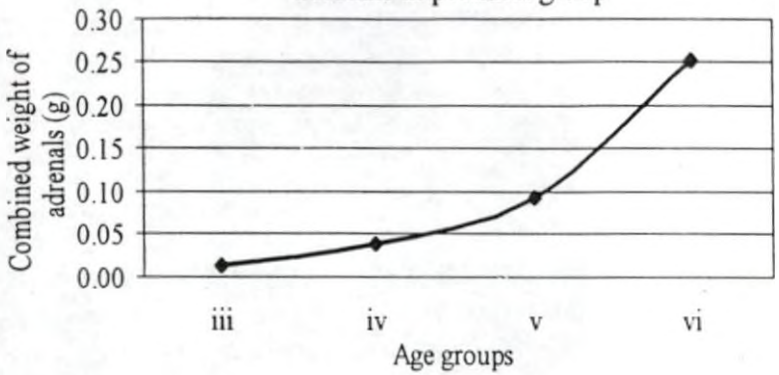


Fig. 4 Relationship between bodyweight and combined weight of adrenals in prenatal group

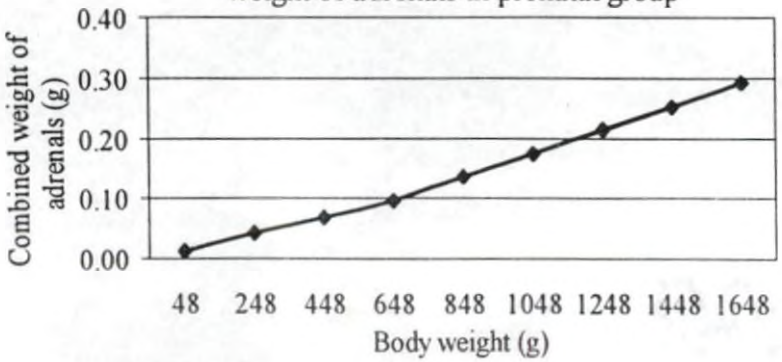


Fig. 5 Relationship between age and combined weight of adrenals in postnatal group

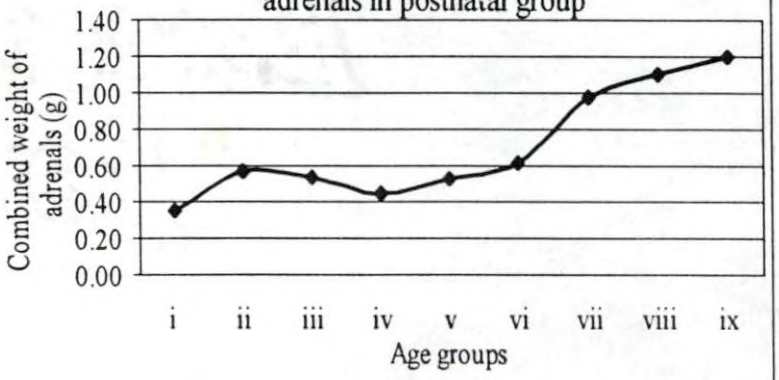


Fig. 6 Relationship between bodyweight and combined weight of adrenals in postnatal group

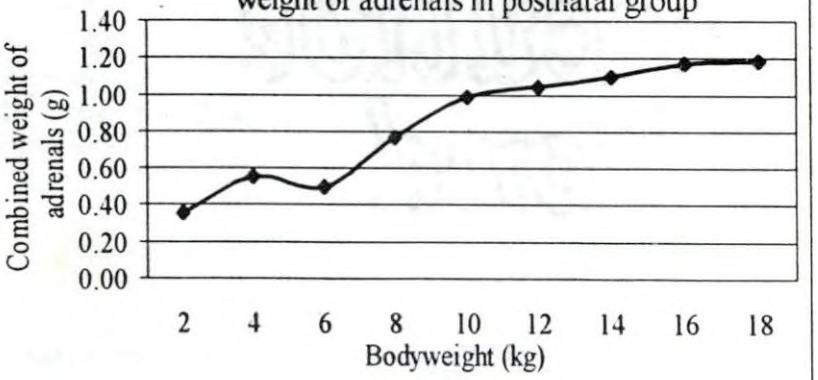


Fig. 7 Percentage contribution of right and left adrenals to the combined adrenal weight in prenatal group

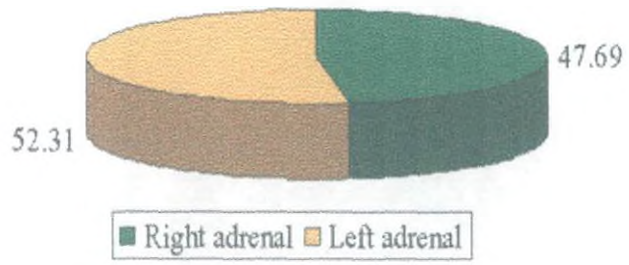


Fig. 8 Percentage contribution of right and left adrenals to the combined adrenal weight in postnatal group

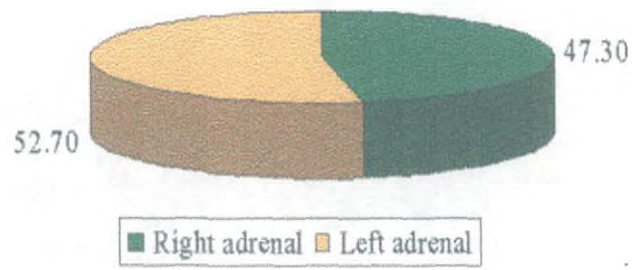


Fig. 9 Relationships between the age and length, breadth and thickness of right adrenal in prenatal group

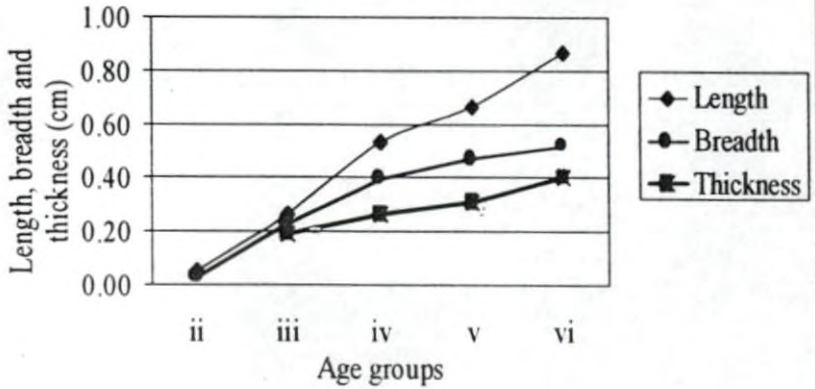


Fig. 10 Relationships between the age and length, breadth and thickness of left adrenal in prenatal group

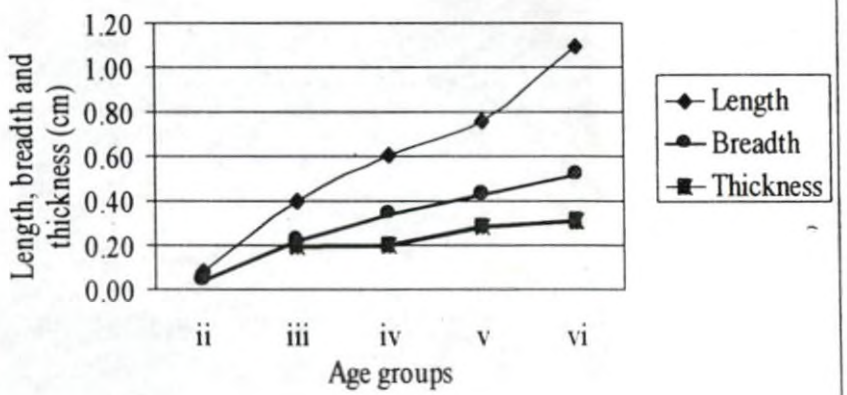


Fig. 11 Relationships between the age and length, breadth and thickness of right adrenal in postnatal group

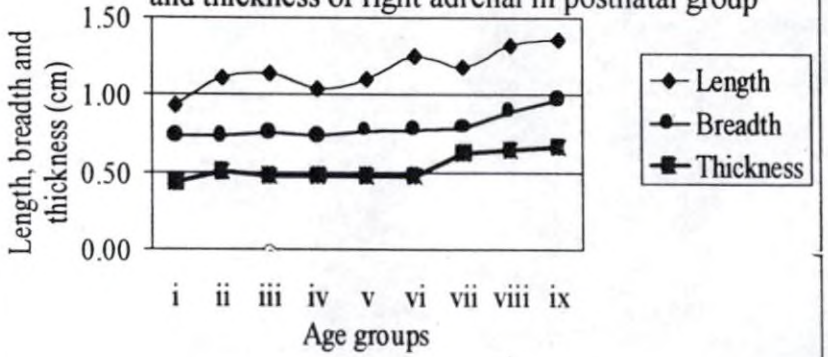


Fig. 12 Relationships between the age and length, breadth and thickness of left adrenal in postnatal group

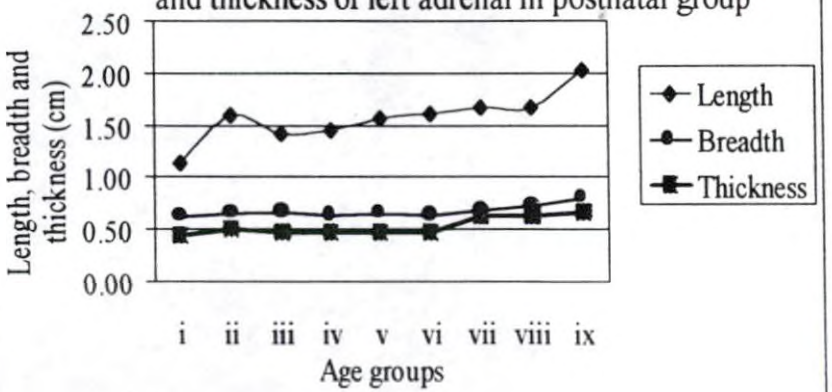


Fig.13 Photograph of a 45 days old kid showing the location of adrenals (arrows)

R - Right adrenal
L - Left adrenal

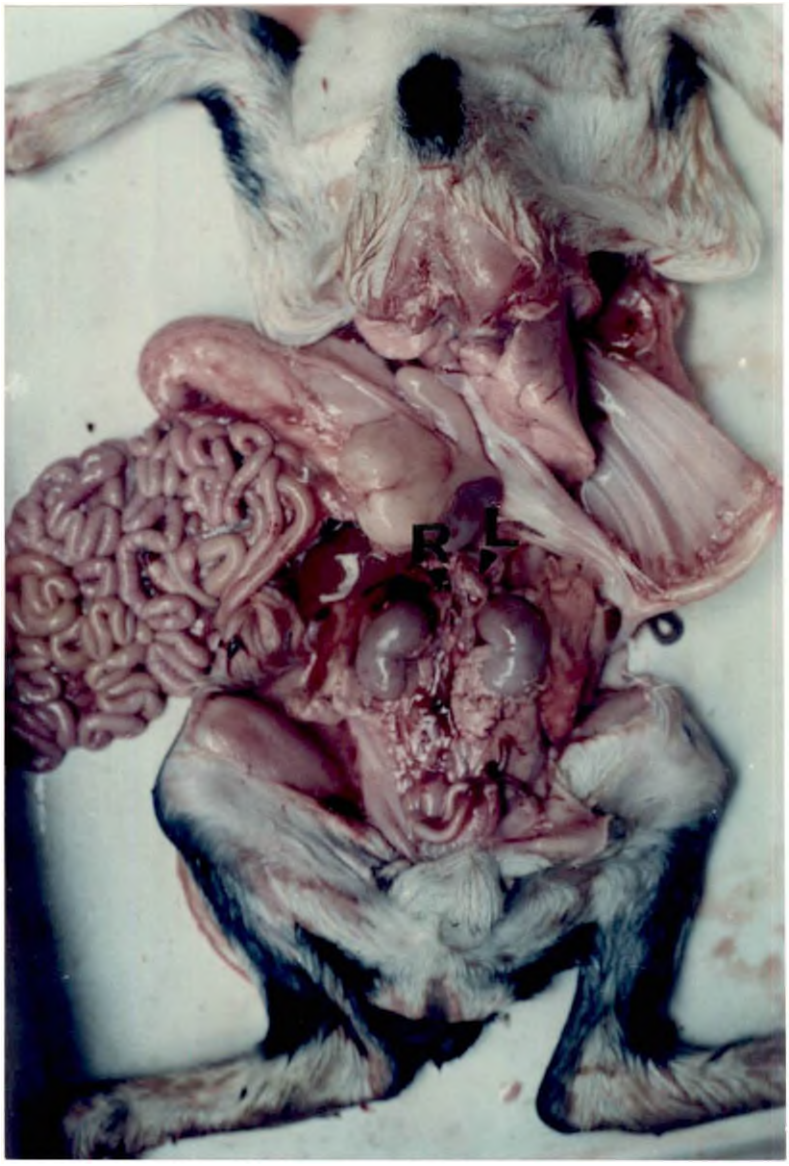

R
L
P

Fig.14 Sagittal section of 33 days embryo showing the formation of cortical primordium. Whorls and clusters of cells (arrows) anterior to the developing metanephros

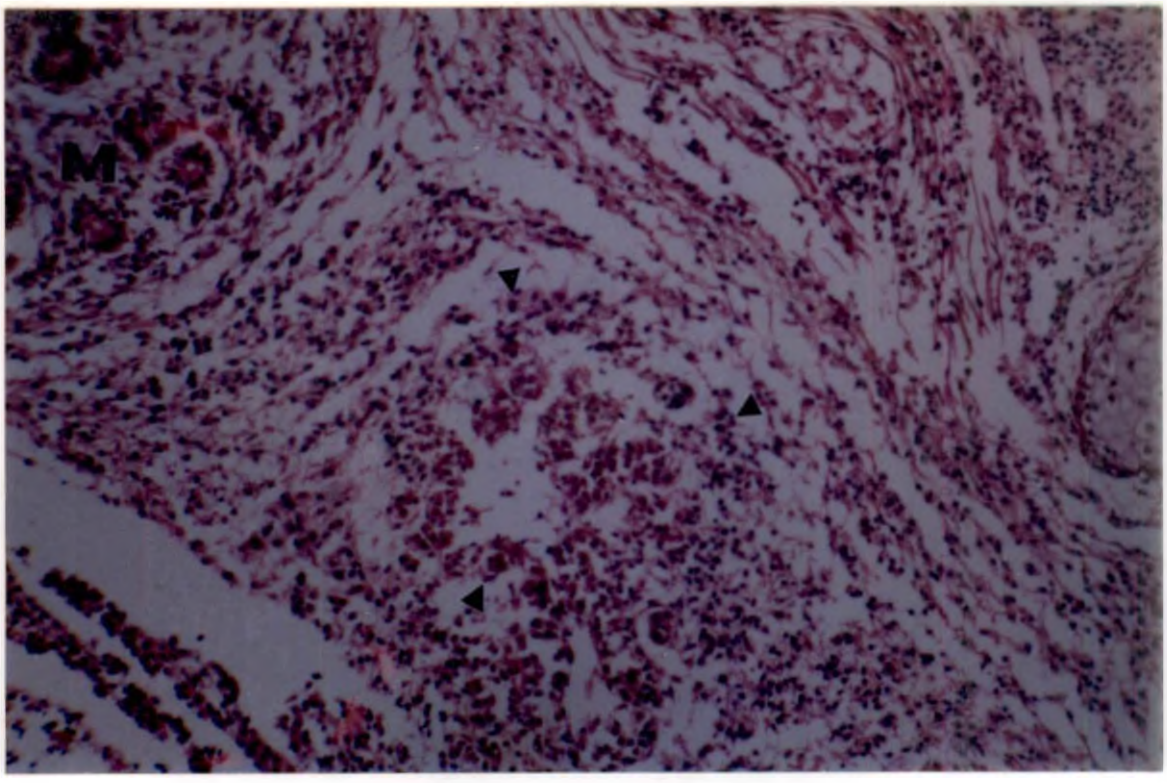
M - Metanephros

H&Ex125

Fig.15 Sagittal section of 42 days foetus showing metanephros (M), adrenal (A) and liver (L)

CV - Central vein
C - Capsule

Masson's trichrome method x 125


M

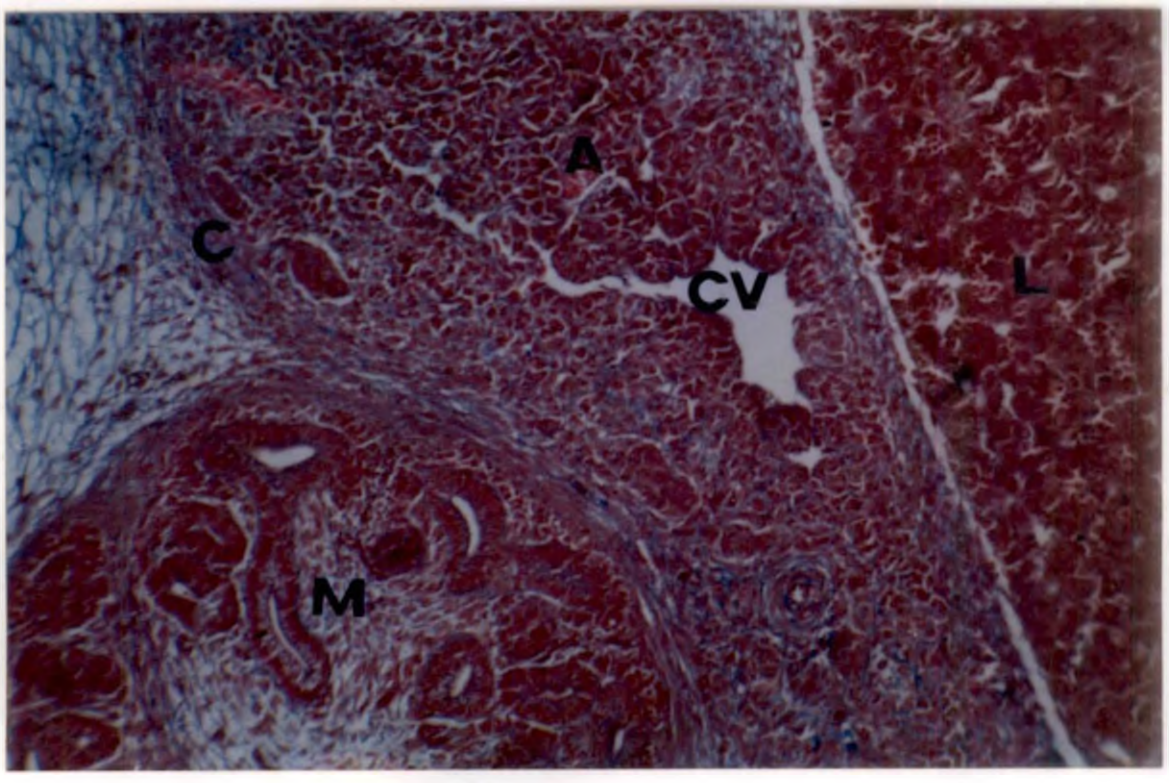

A
C
CV
L
M

Fig.16 Section of adrenal (50 days foetus) showing the capsule and aggregations of cortical and migratory cells

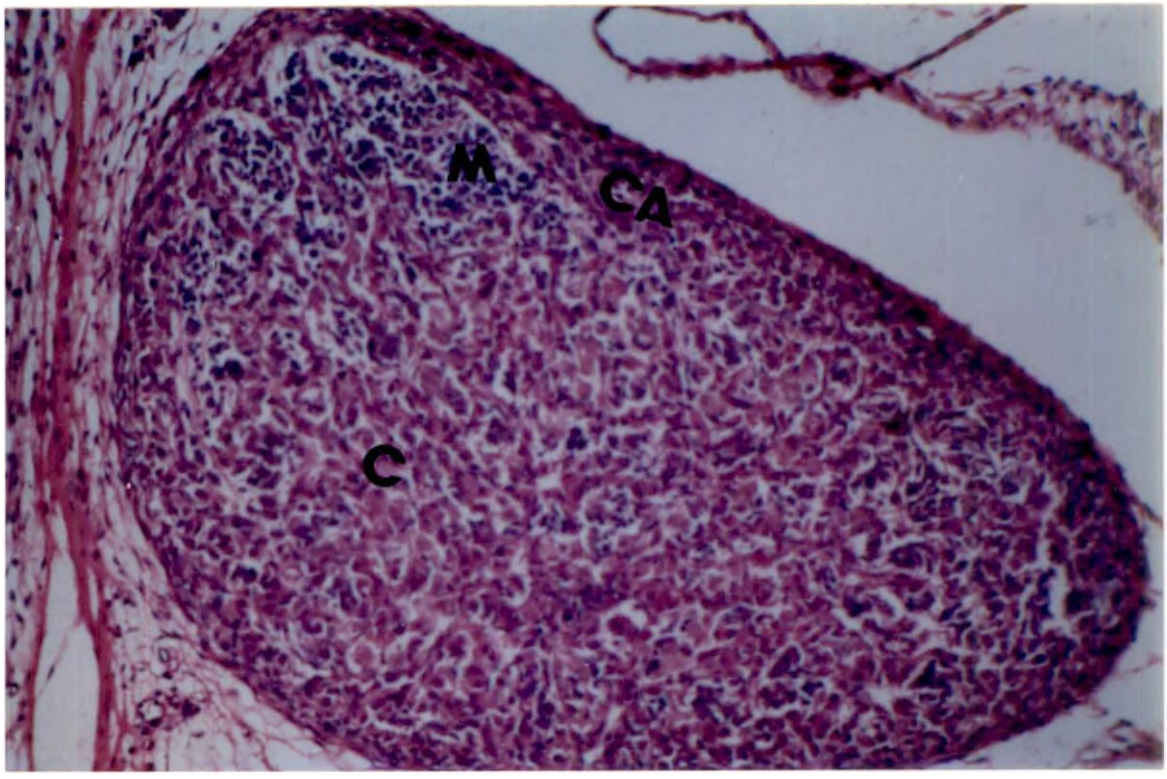
CA - Capsule
C - Cortical cells
M - Migratory cells

H&Ex125

Fig.17 Section of adrenal (50 days foetus) showing migratory cells in the capsule

C - Cortical cells
M - Migratory cells

H&Ex125


M
CA
C

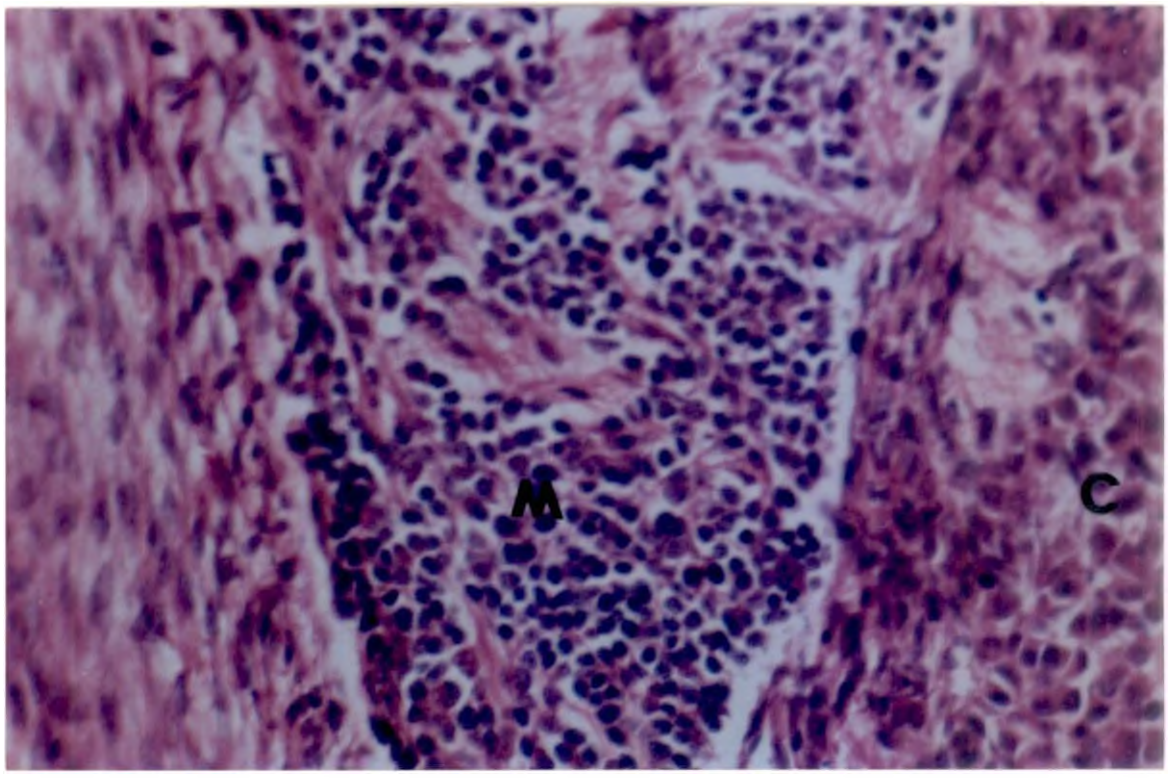
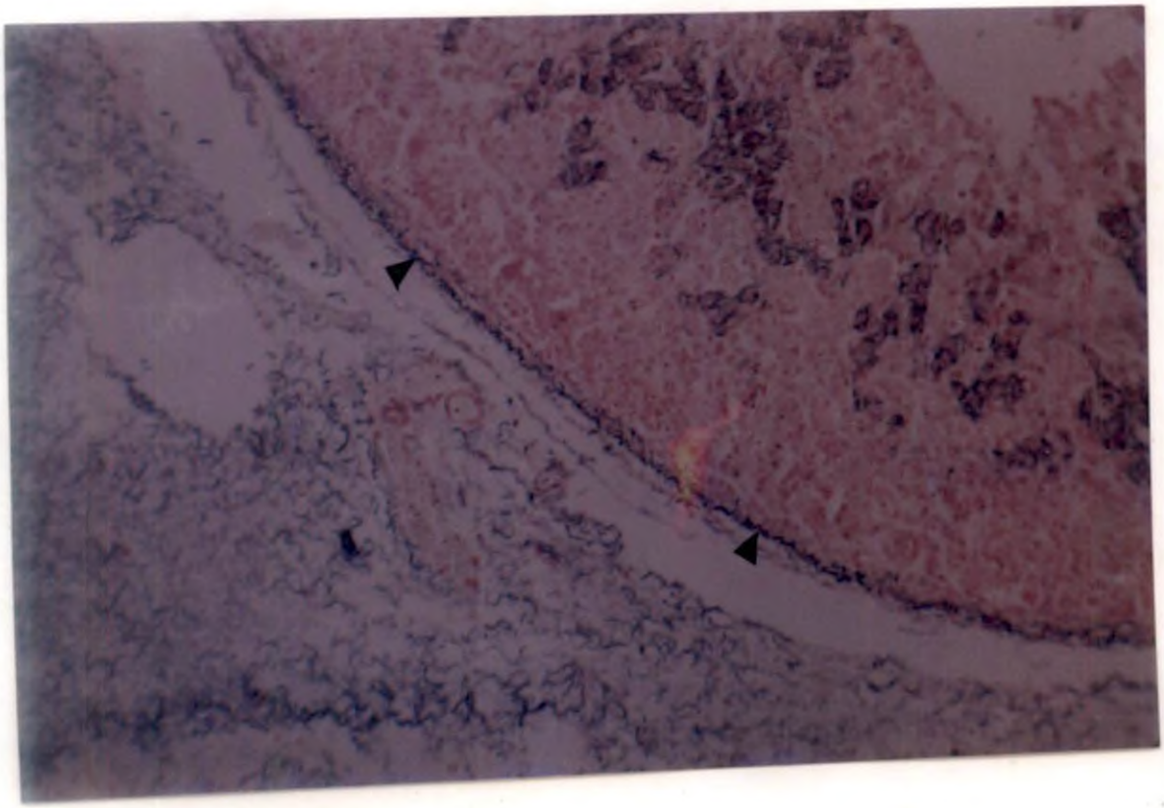

M
C

Fig.18 Section of adrenal (50 days foetus) showing mild chromaffin reaction (arrows)

H&Ex125

Fig.19 Section of adrenal (58 days foetus) showing reticular fibres in the capsule (arrows)

Gridley's method x 125



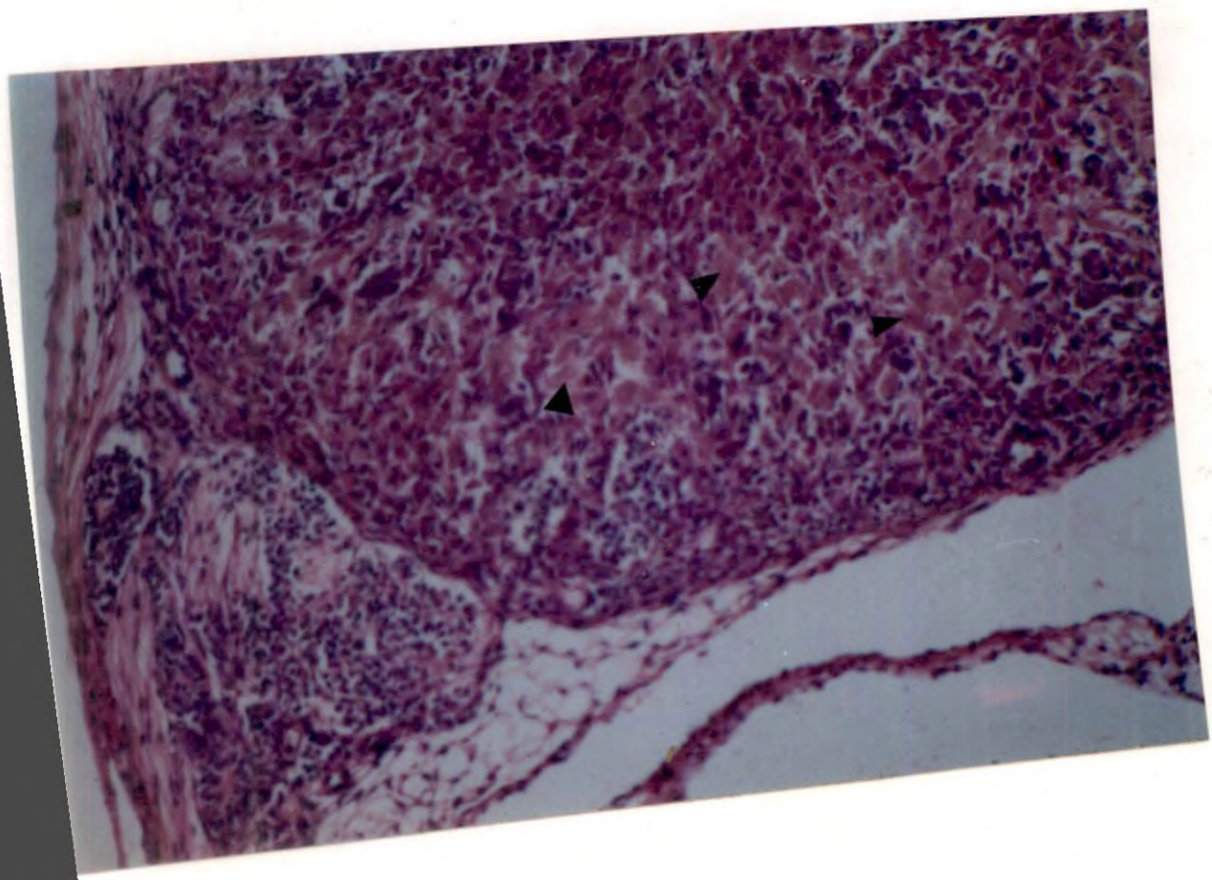
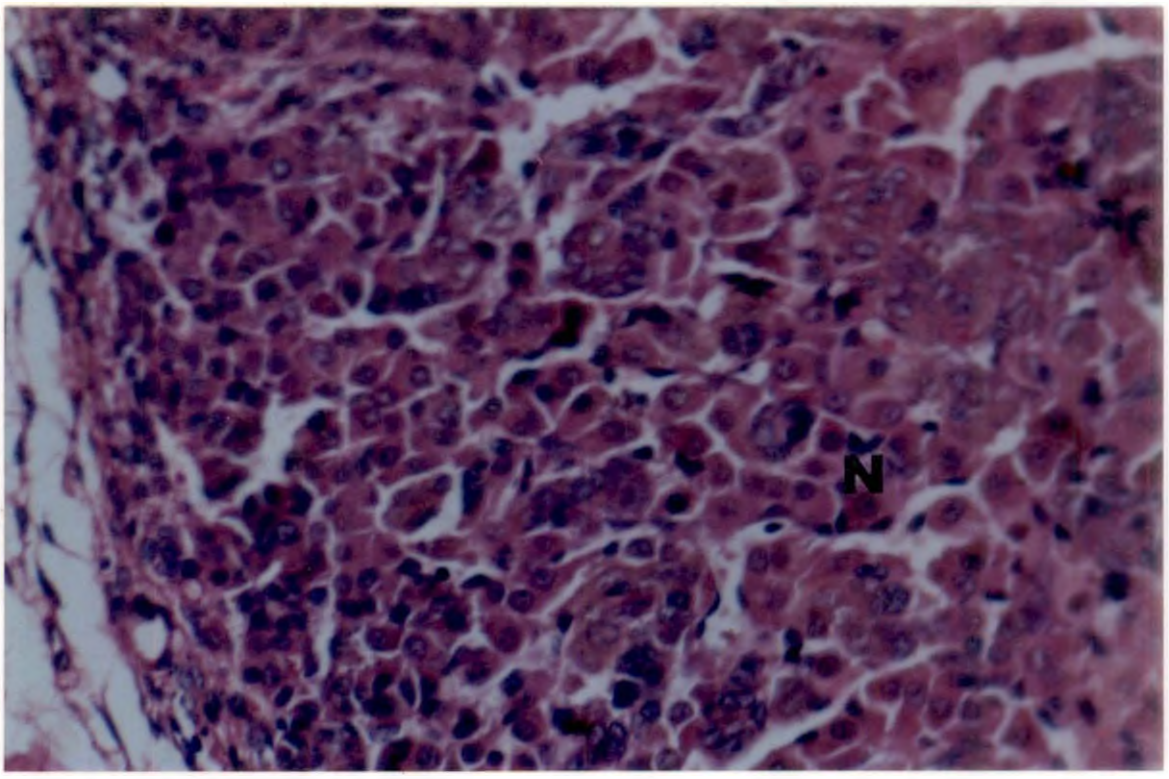


Fig.20 Section of adrenal (62 days foetus) showing cells with neuronal characters (N)

H&Ex312.5

Fig.21 Section of adrenal (70 days foetus) showing the fibrous (F) and cellular (C) layers of capsule and corticomedullary separation

H&Ex125


N

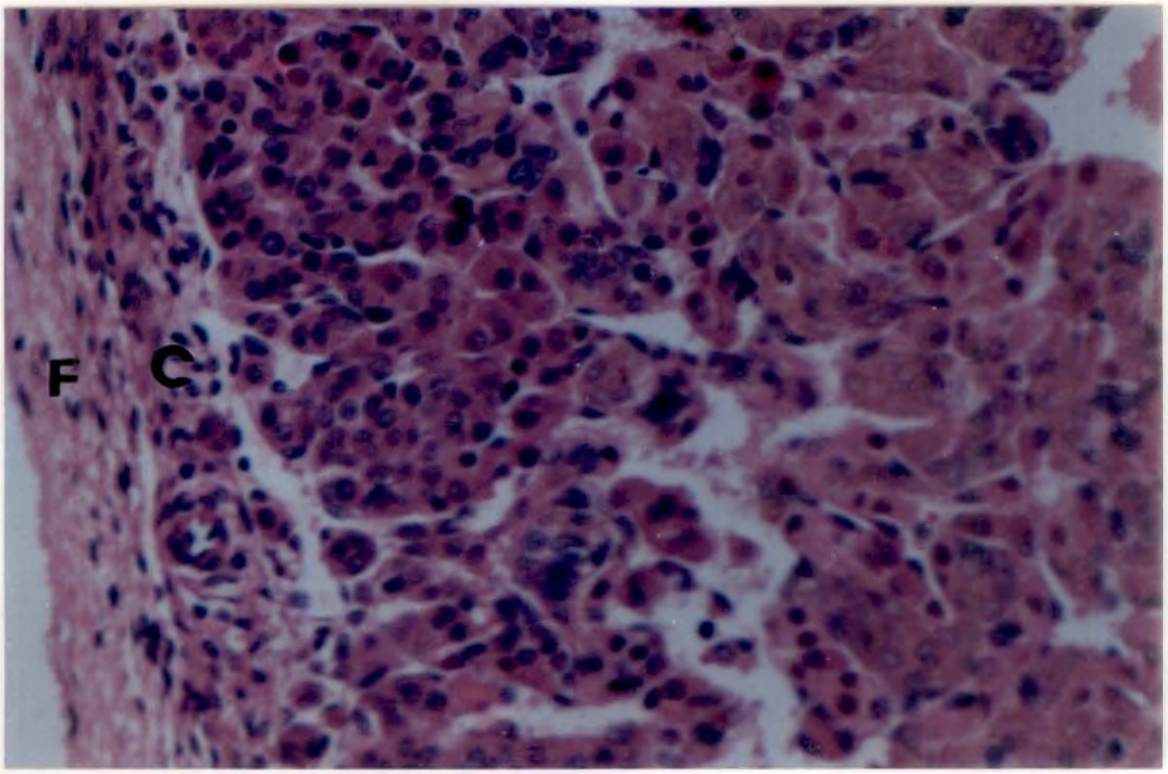
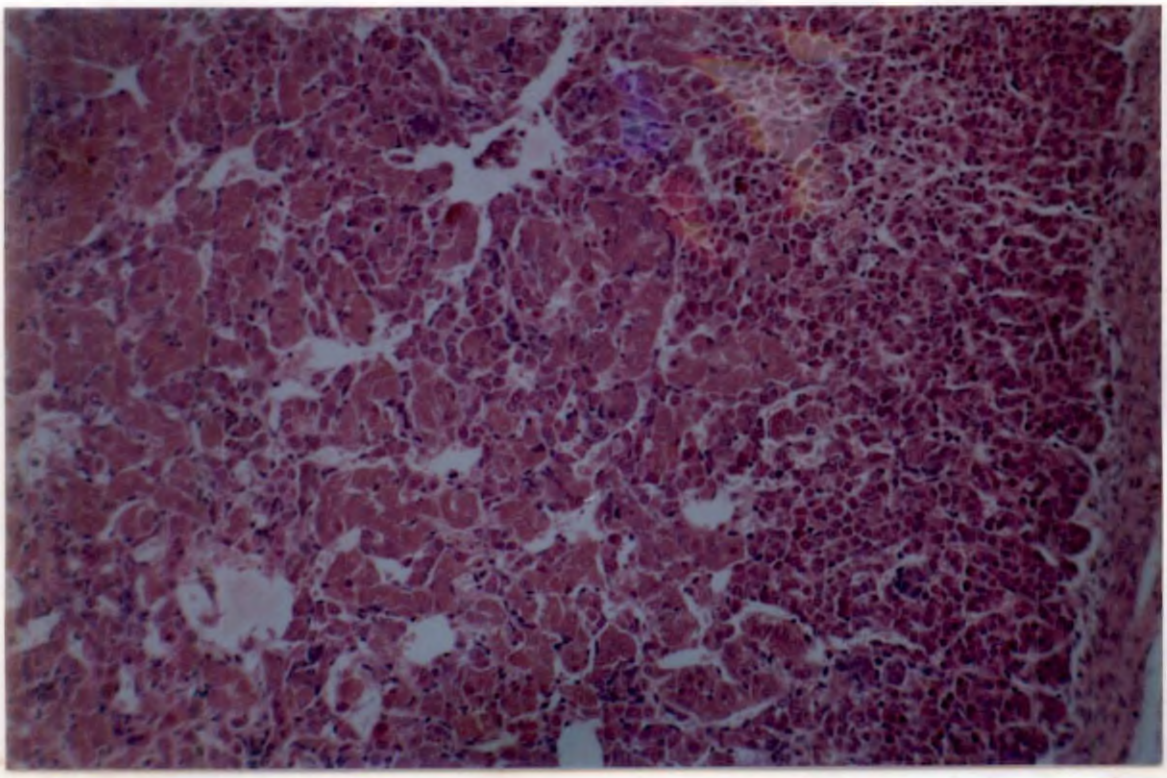

F
C

Fig.22 Section of adrenal (74 days foetus) showing organisation of cell clusters beneath the capsule

H&Ex312.5

Fig.23 Section of adrenal (74 days foetus) showing mixture of cortical and chromaffin cells at the centre

H&Ex125



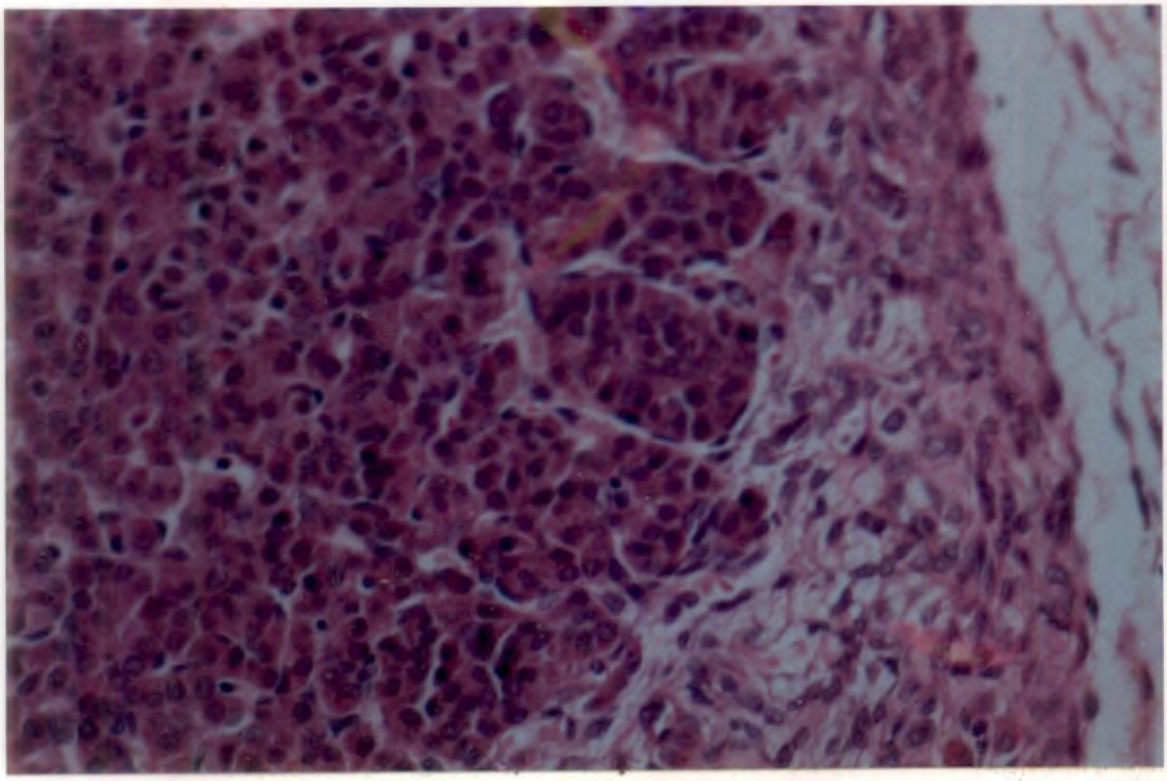
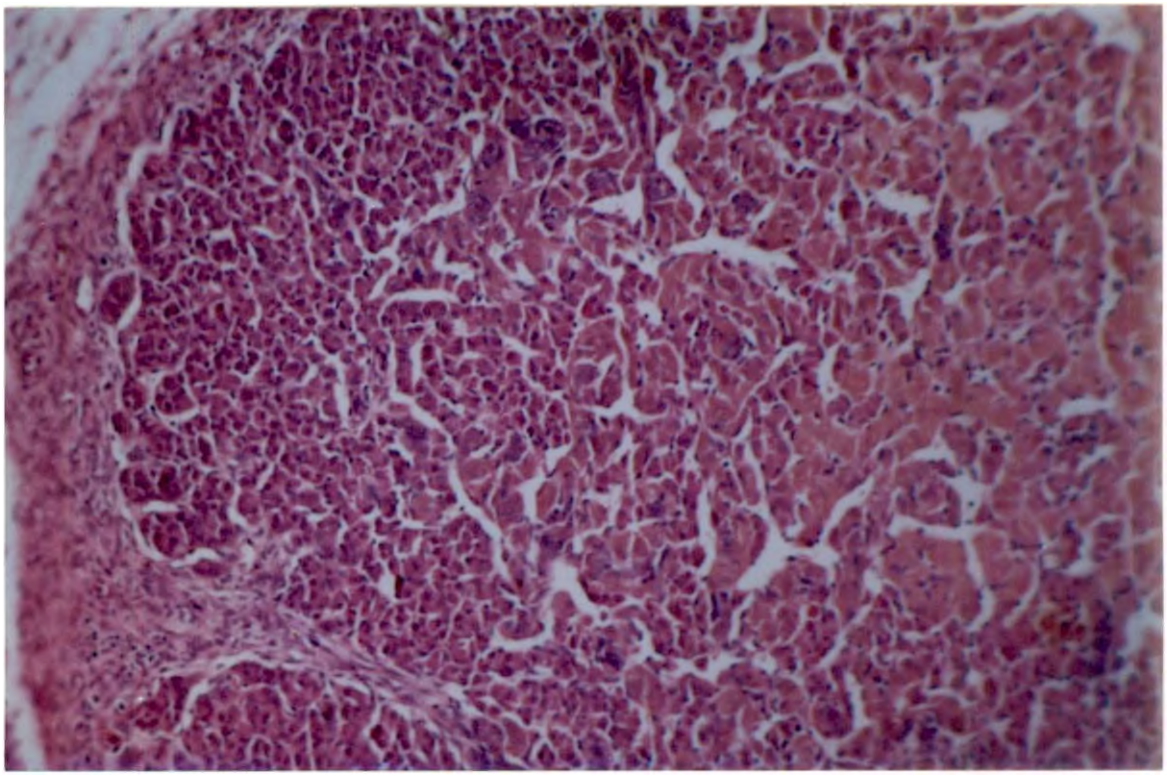


Fig.24 Section of adrenal (78 days foetus) showing well developed capsule and trabeculae

H&Ex125

Fig.25 Section of adrenal (98 days foetus) showing concentration of chromaffin cells at the centre H&Ex125



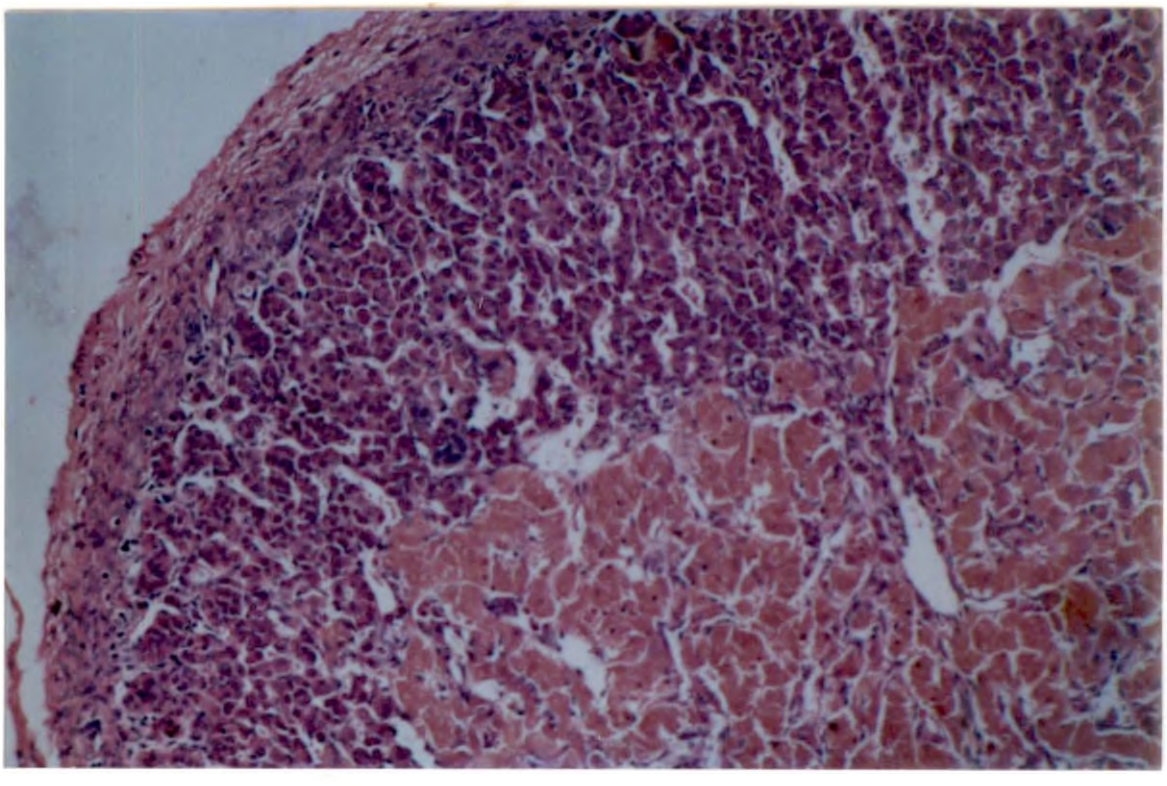


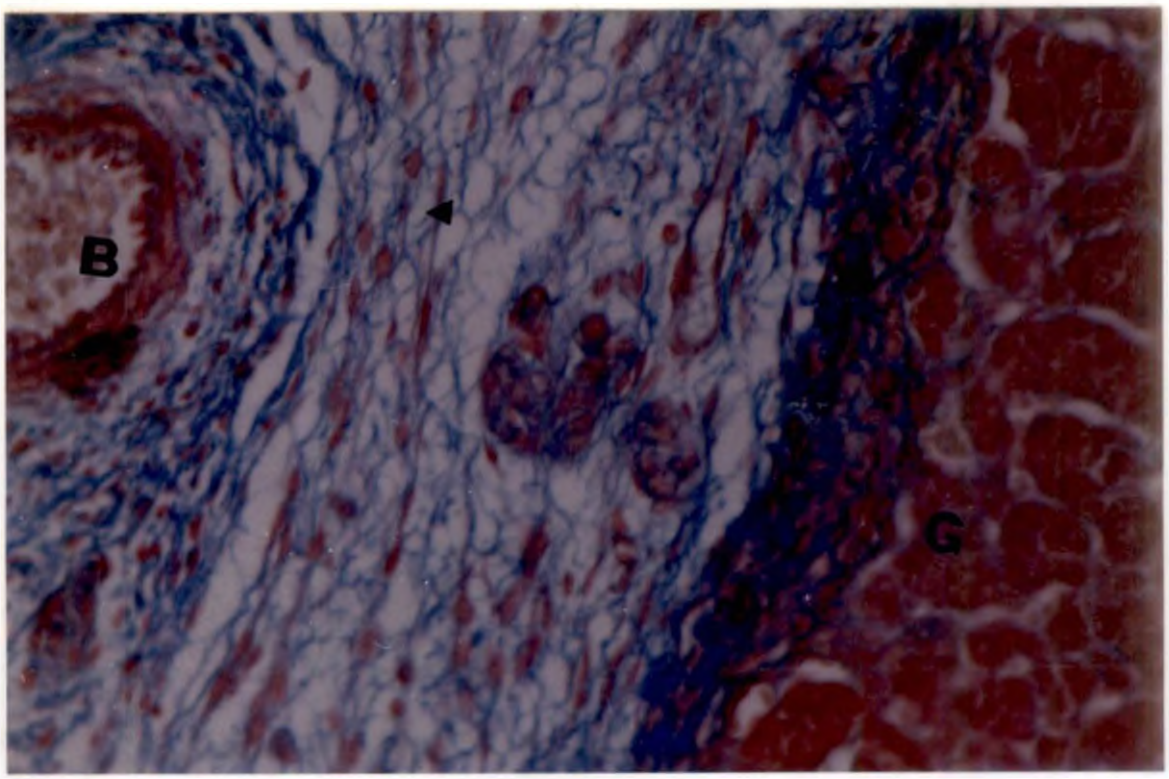
Fig.26 Section of adrenal (101 days foetus) showing smooth muscle cells (arrow) and collagen fibres in the capsule

B - Blood vessel
G - Zona glomerulosa

Masson's trichrome method x 312.5

Fig.27 Section of adrenal (107 days foetus) showing highly vascular capsule with numerous trabeculae

Mallory's PTAH method x 125


B
G

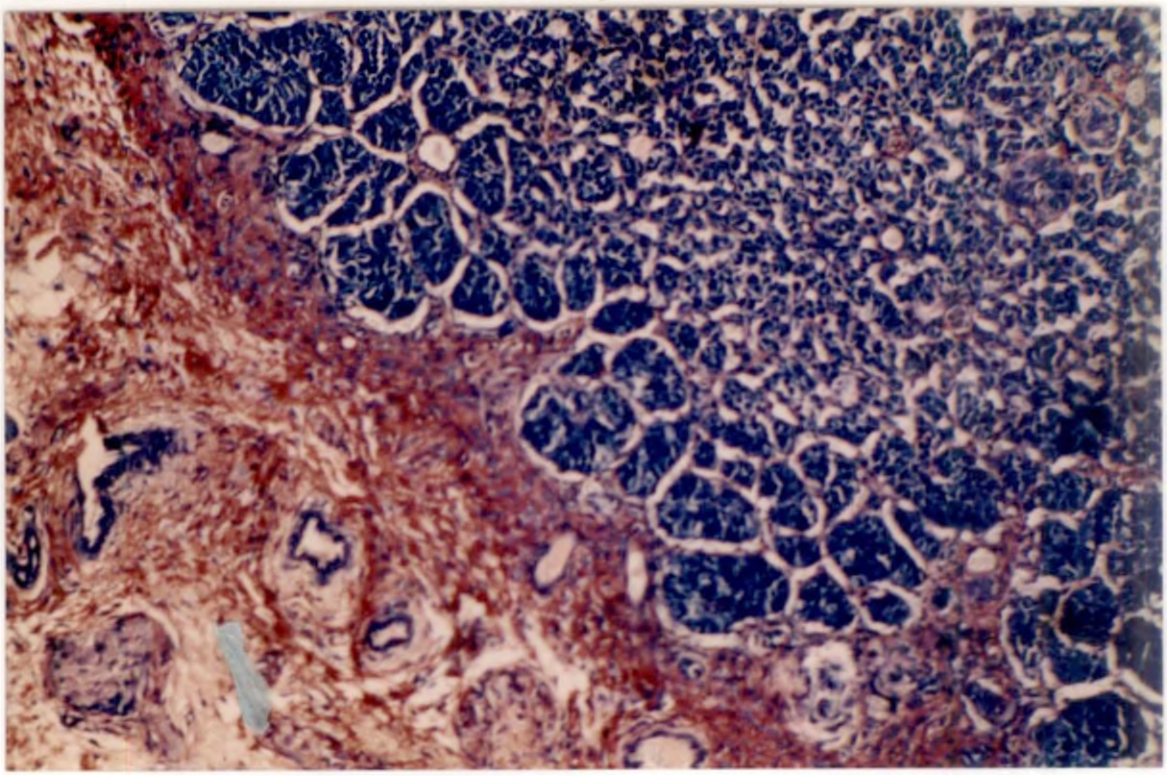
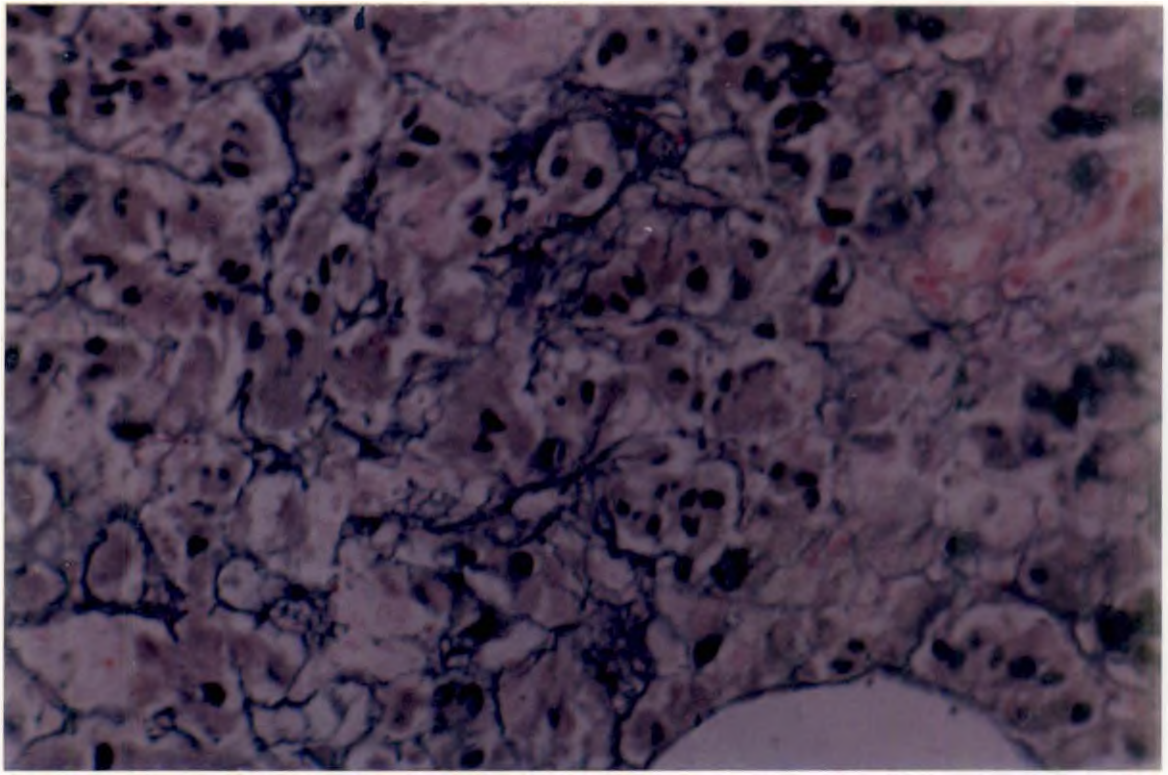


Fig.28 Section of adrenal (107 days foetus) showing reticular fibres surrounding medullary cells

Gridley's method x 312.5

Fig.29 Section of adrenal (122 days foetus) showing well differentiated zona glomerulosa and developing deeper zones

H&Ex125



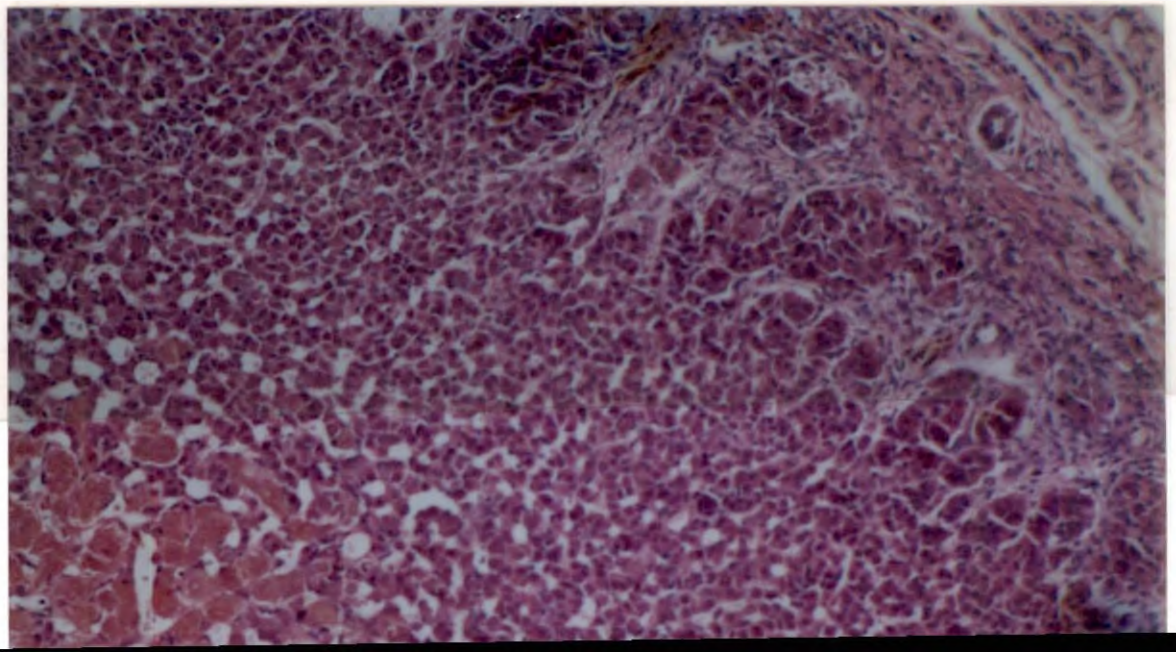


Fig.30 Section of adrenal (129 days foetus) showing well differentiated cortical zones

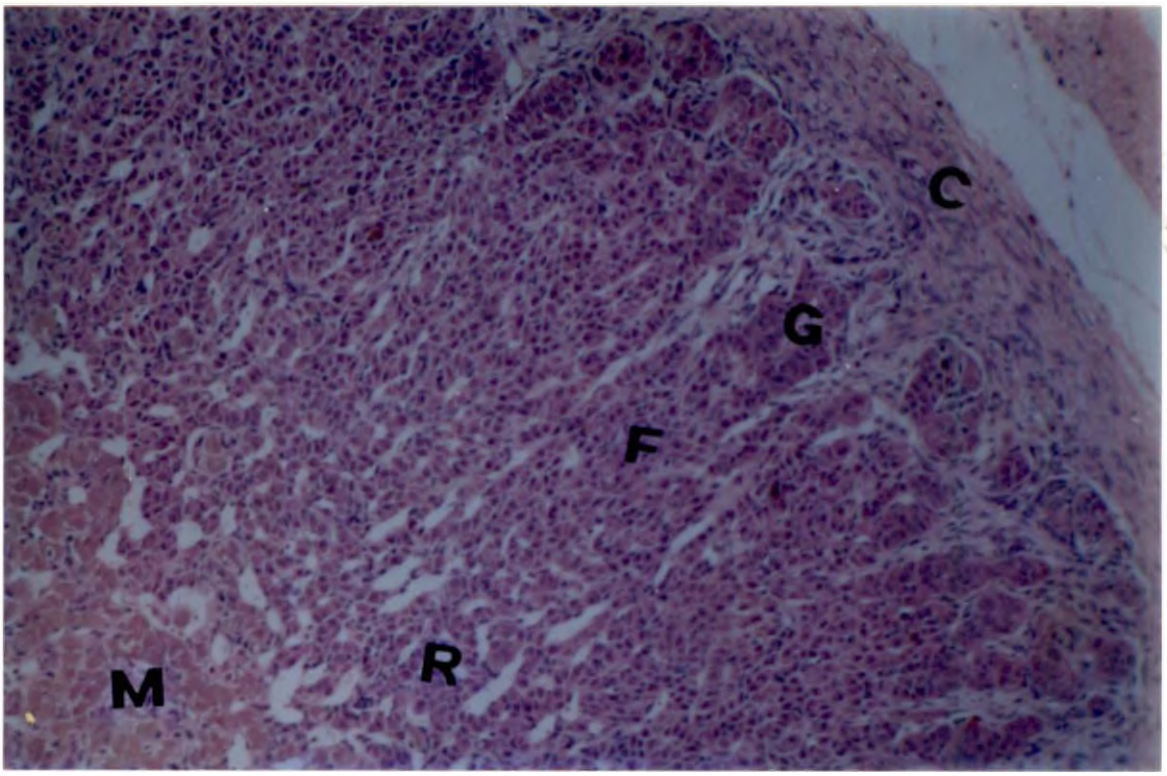
C - Capsule
G - Zona glomerulosa
F - Zona fasciculata
R - Zona reticularis
M - Medulla

H&Ex125

Fig.31 Section of adrenal (138 days foetus) showing cortical cells around the central vein

CV - Central vein
C - Cortical cells
M - Medullary cells

H&Ex312.5


C
G
F
M
R

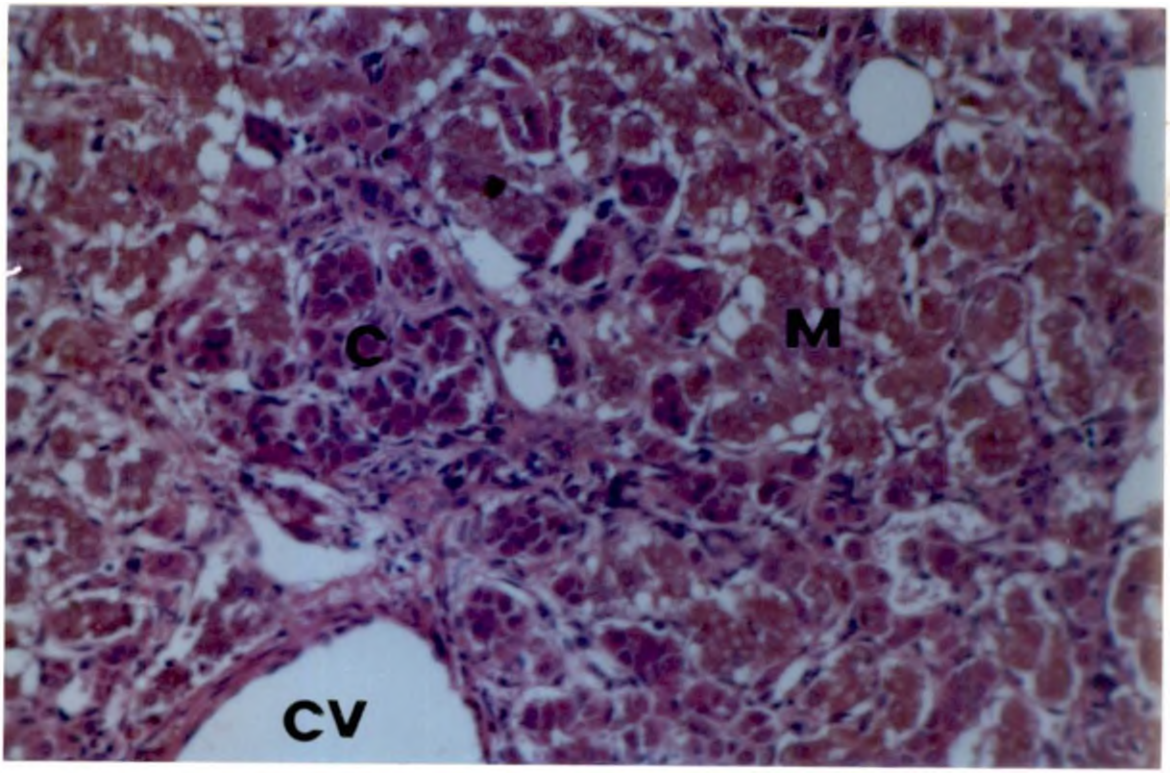

C
M
CV

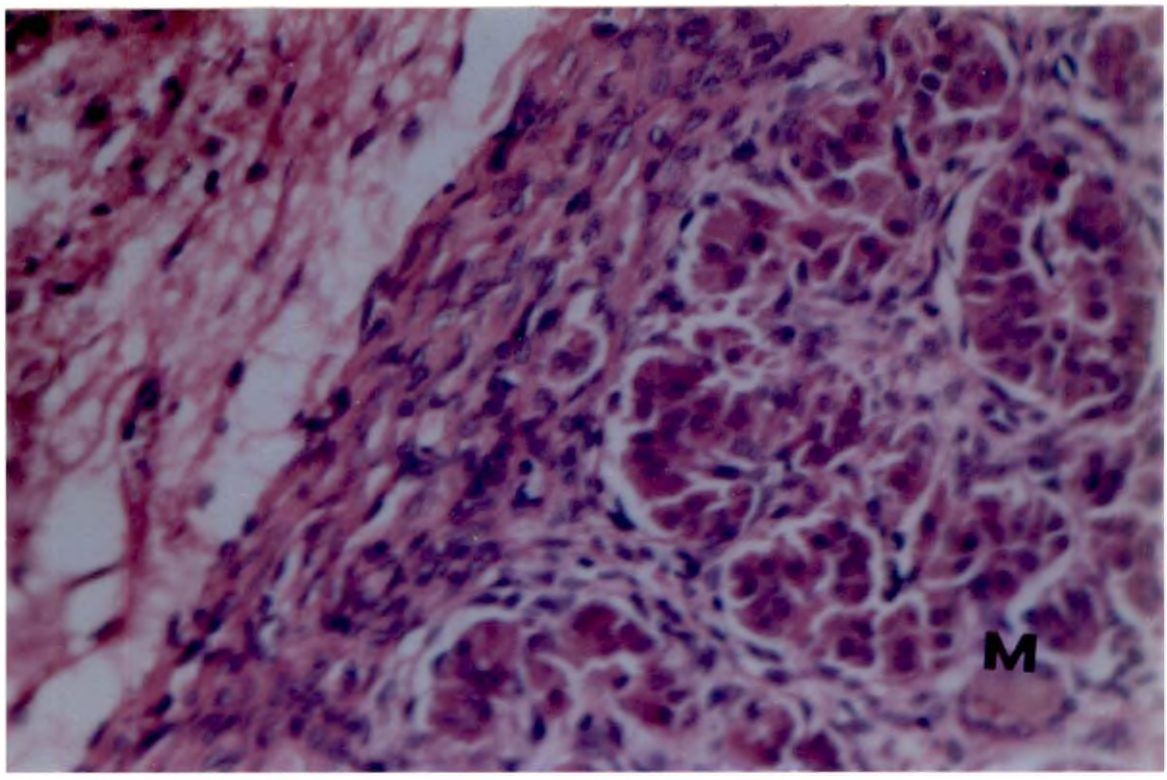
Fig.32 Section of adrenal (129 days foetus) showing glomerulosa cells in clusters

M - Medullary cell trapped in the cortex

H&Ex312.5

Fig.33 Section of adrenal medulla (full term foetus) showing follicles containing colloid material

H&Ex312.5


M

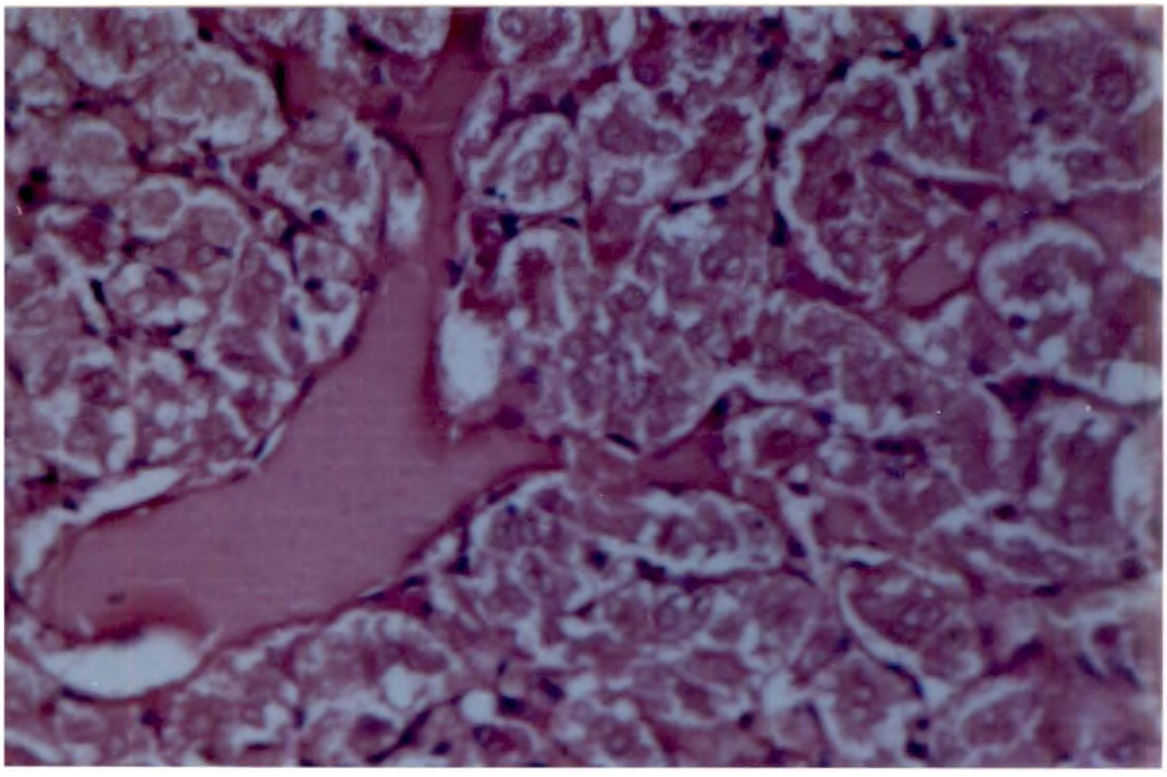
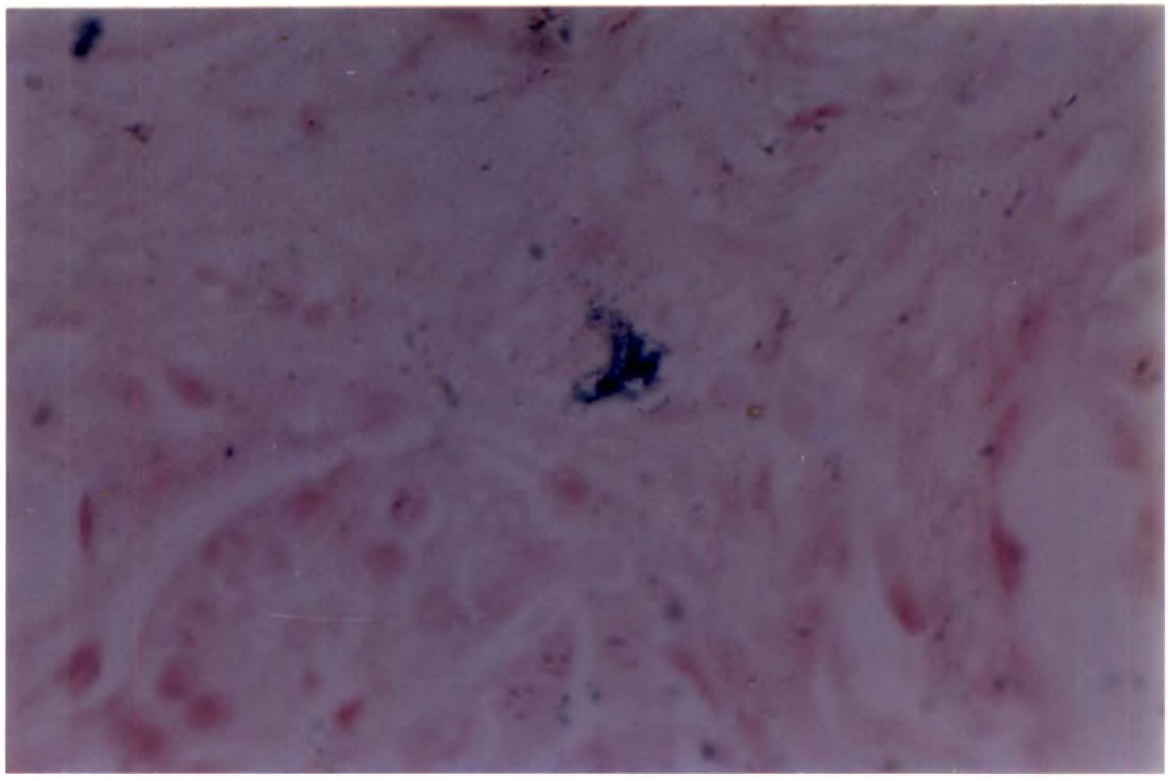


Fig.34 Section of adrenal (15 days - postnatal) showing melanocyte in the capsule

Fontana-Masson's silver method x 787.5

Fig.35 Section of adrenal (15 days - postnatal) showing a ganglion in the capsule

H&Ex312.5



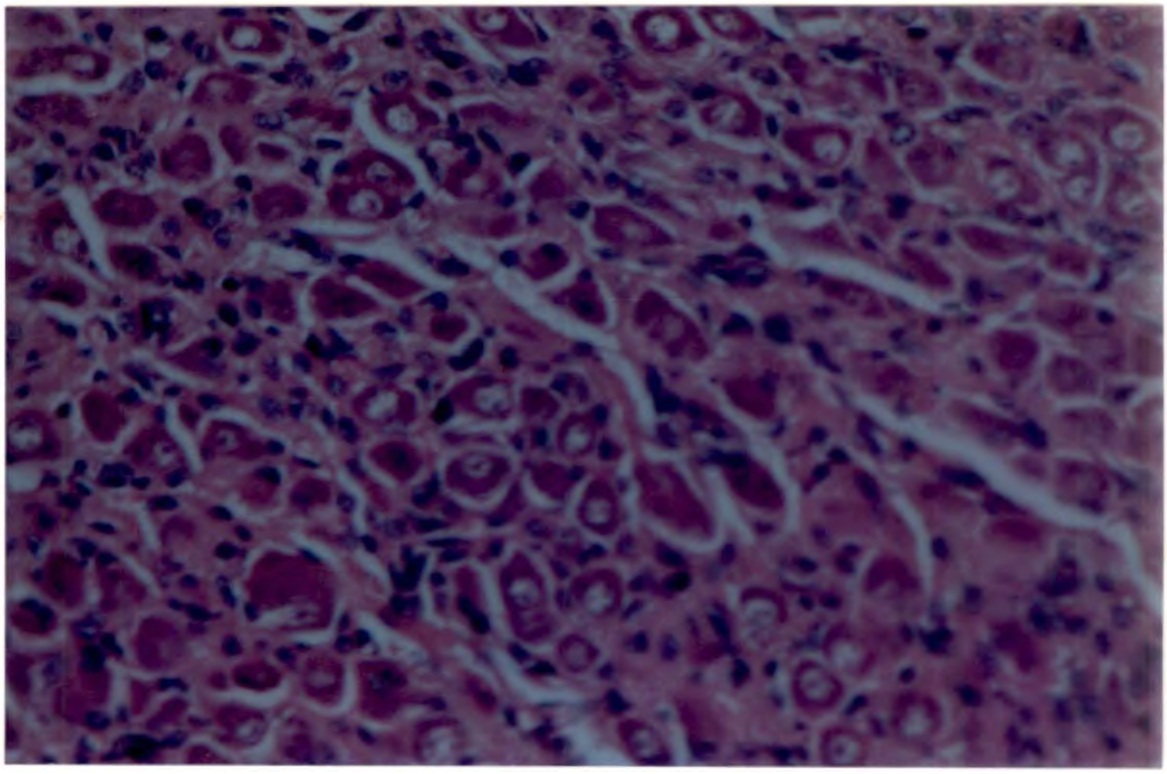


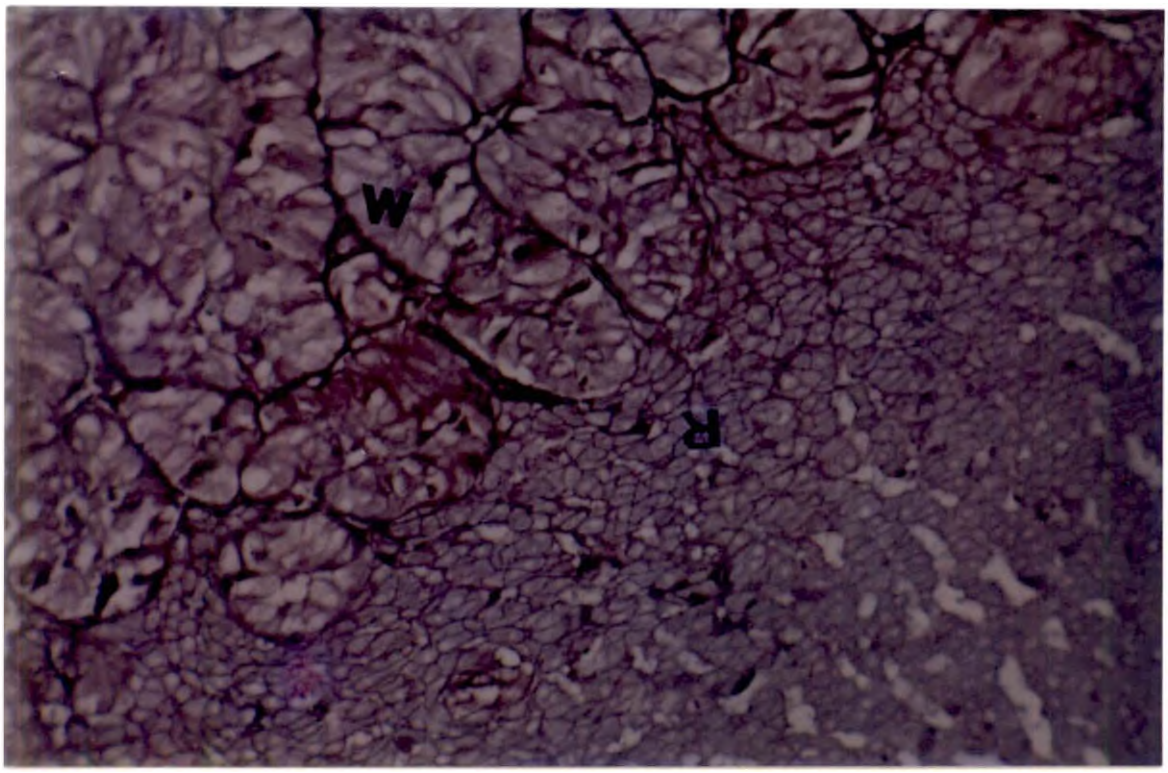
Fig.36 Section of adrenal (15 days - postnatal) showing groups of cortical cells in the capsule

H&Ex125

Fig.37 Section of adrenal (60 days - postnatal) showing interdigitations at the corticomedullary junction. Note the reticular fibres in the zona reticularis and medulla

R - Zona reticularis
M - Medulla

Gridley's method x 200


W
R

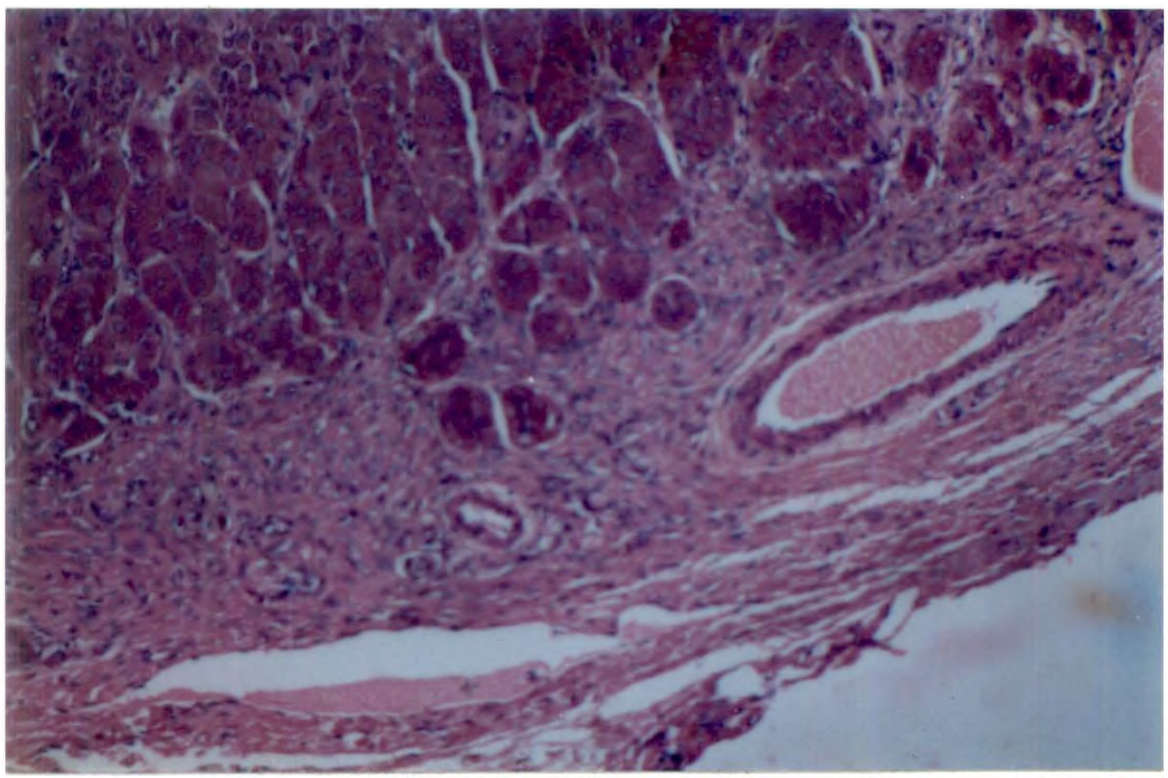
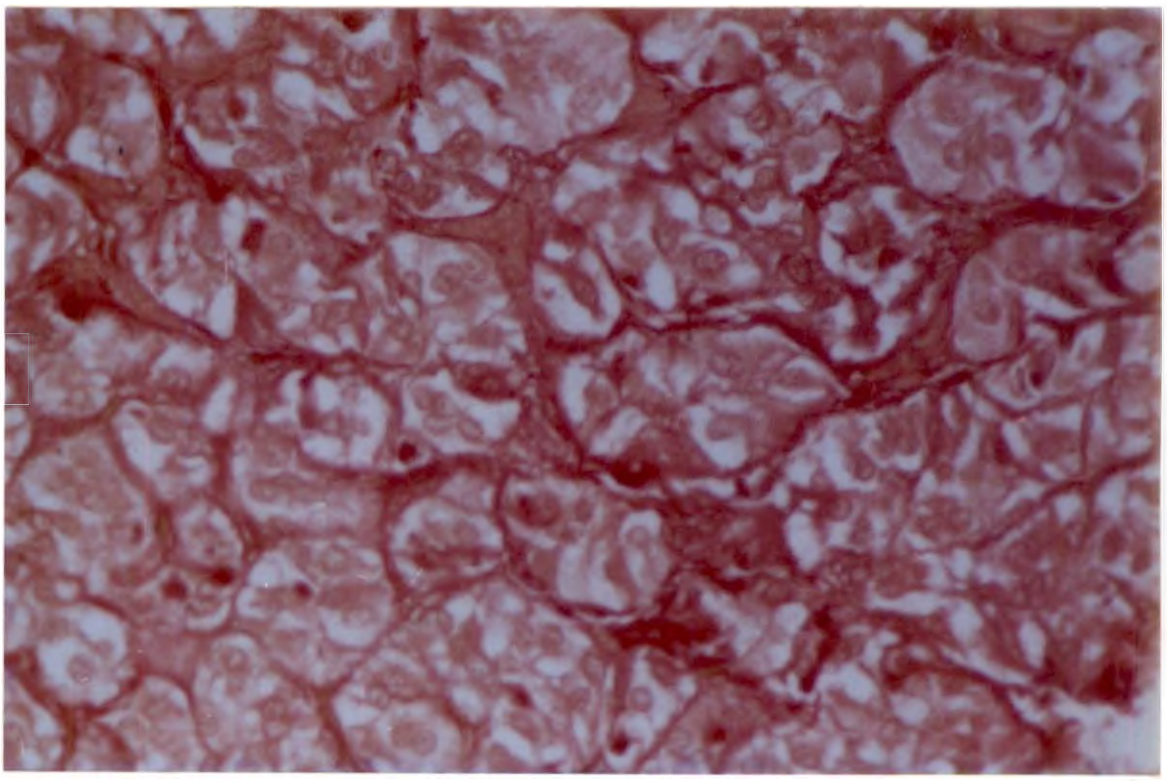


Fig.38 Section of adrenal (15 days - postnatal) showing alveolar arrangement of collagen in the medulla

Van Gieson's method x 312.5

Fig.39 Section of adrenal (180 days - postnatal) showing mast cell in the zona fasciculata

Unna's method x 787.5



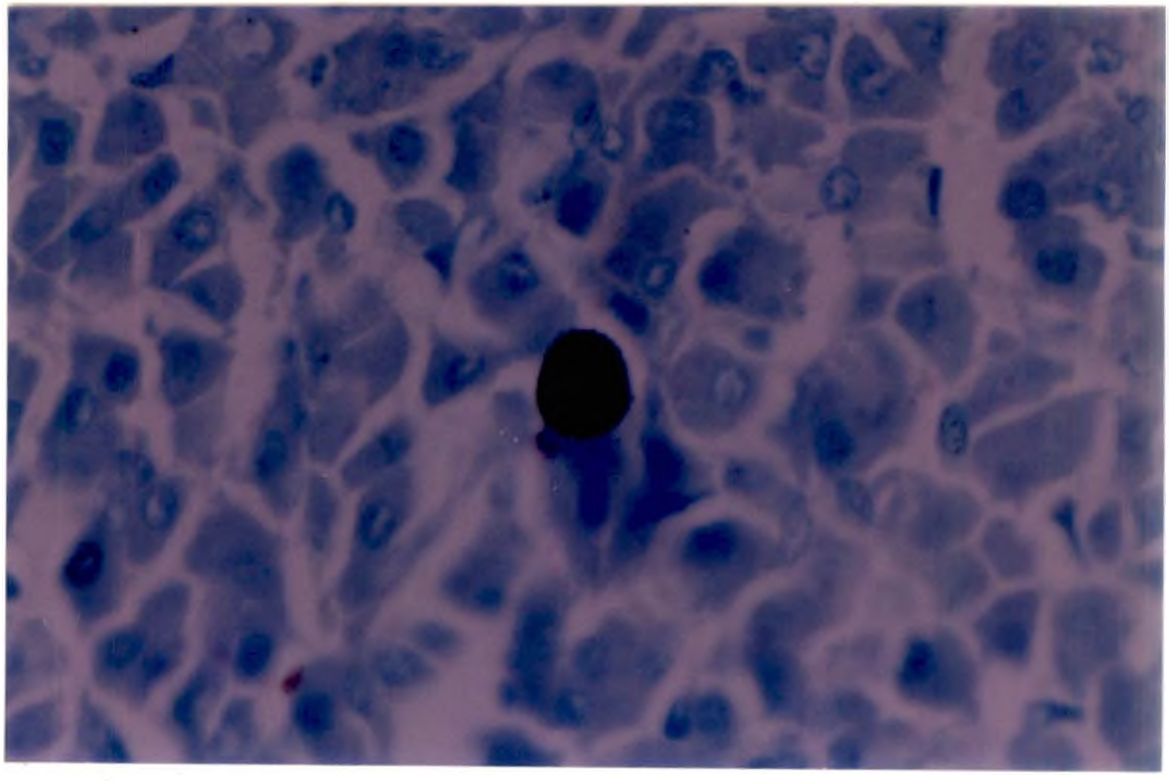


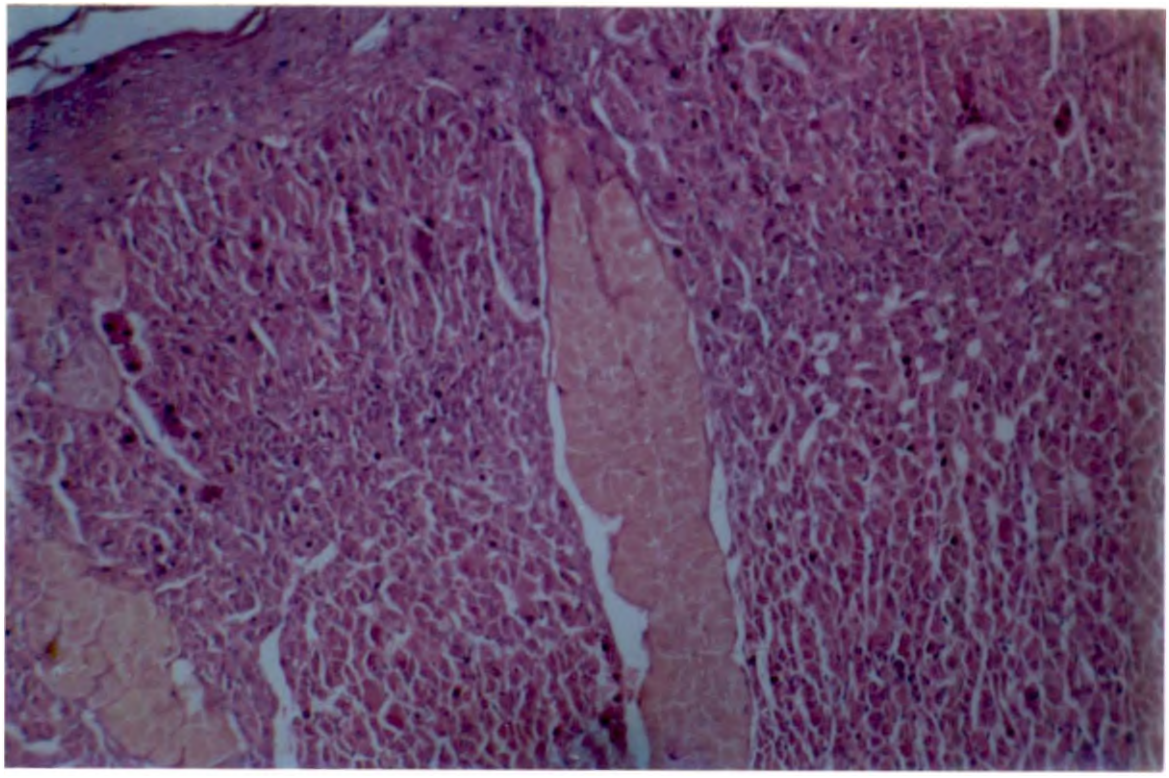
Fig.40 Section of adrenal (180 days - postnatal) showing extensions of medullary cells into the cortex

H&Ex125

Fig.41 Section of adrenal (150 days - postnatal) showing zona intermedia with tightly packed cells

G - Zona glomerulosa
I - Zona intermedia
F - Zona fasciculata

H&Ex125



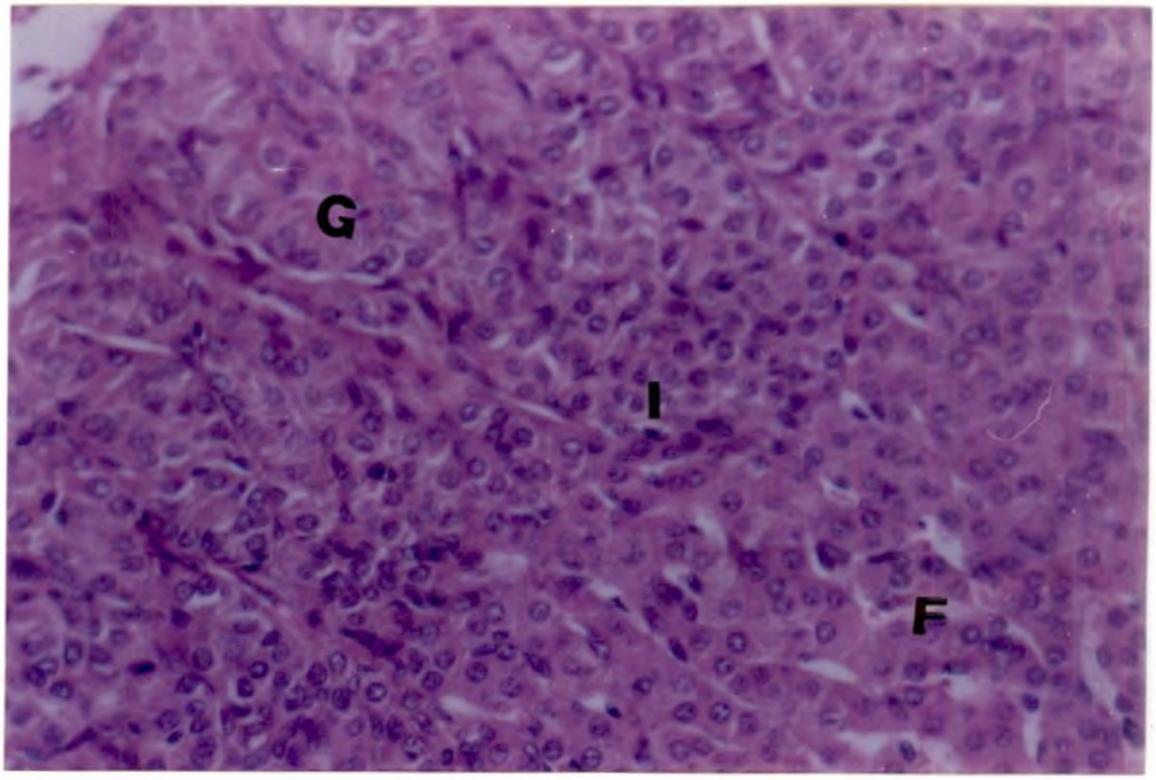
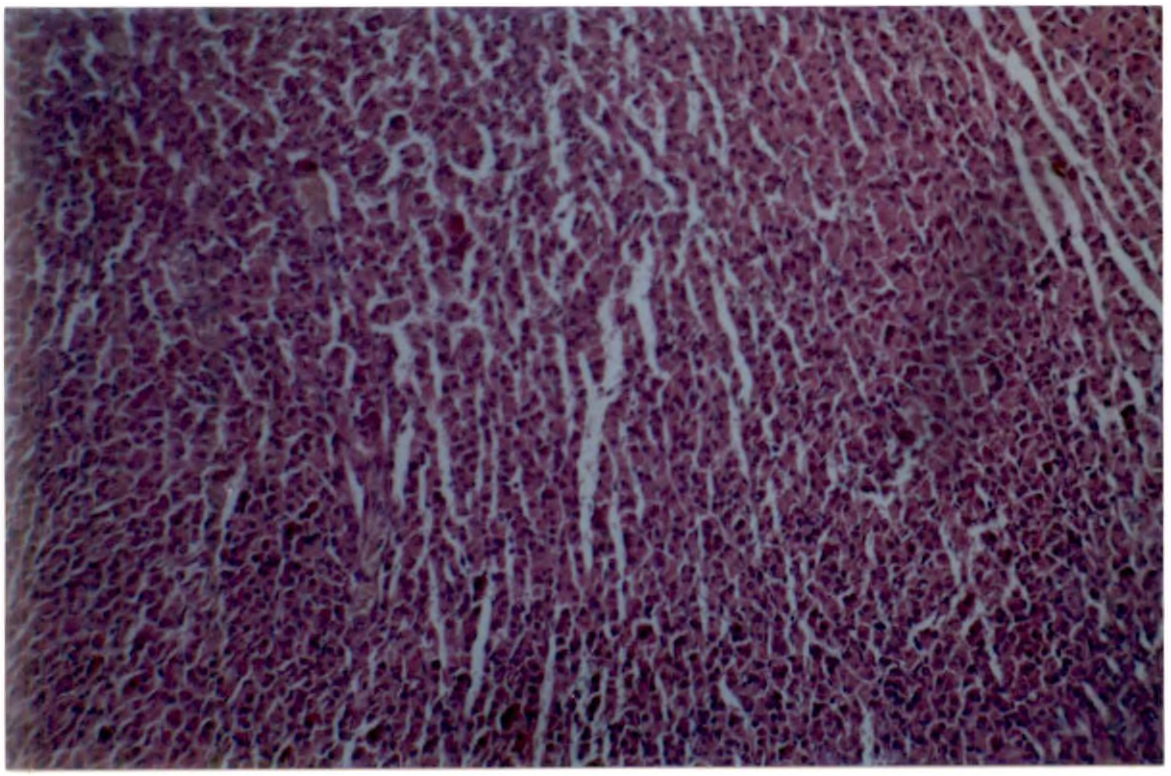

G
I
F

Fig.42 Section of adrenal (90 days - postnatal) showing increased acidophilia towards the inner zone of the cortex

H&Ex125

Fig.43 Section of adrenal (60 days - postnatal) showing arrangement of cells in the zona fasciculata

H&Ex125



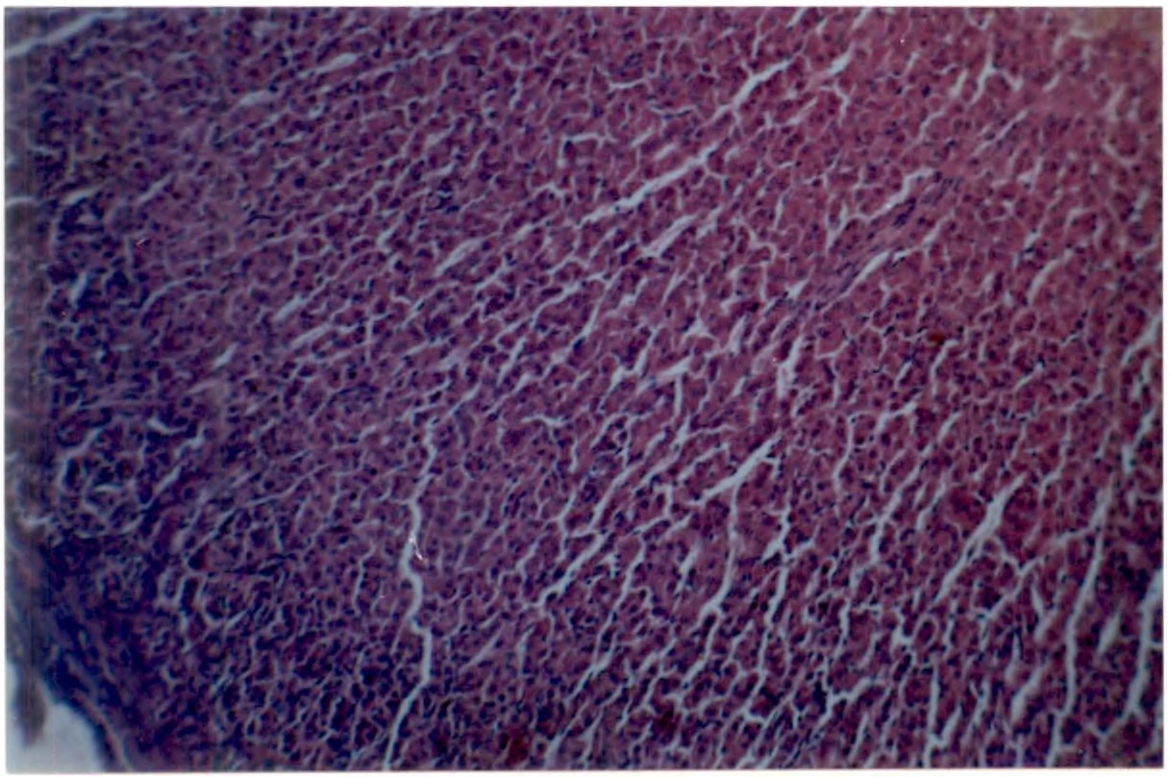
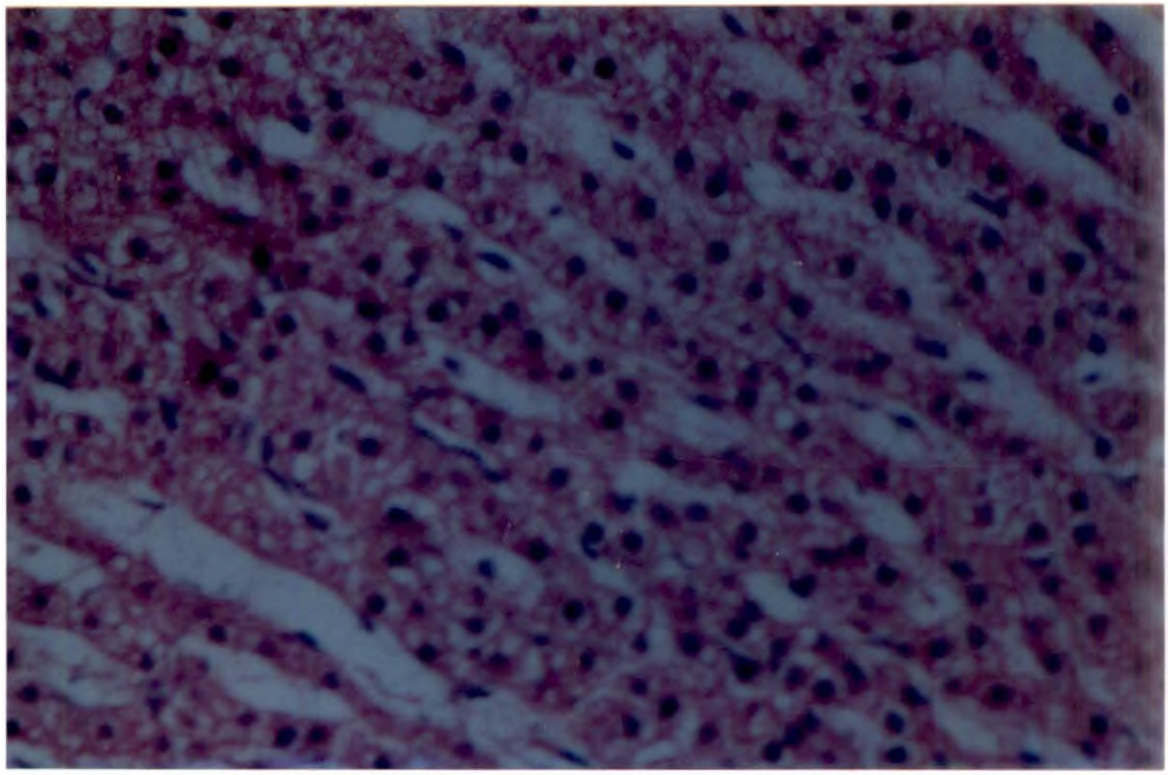


Fig.44 Section of adrenal (30 days - postnatal) showing vacuolated cells of the outer fasciculata

H&Ex312.5

Fig.45 Section of adrenal (60 days - postnatal) showing arrangement of cells in the zona reticularis. Note medullary cells (M) trapped in the zone

H&Ex312.5



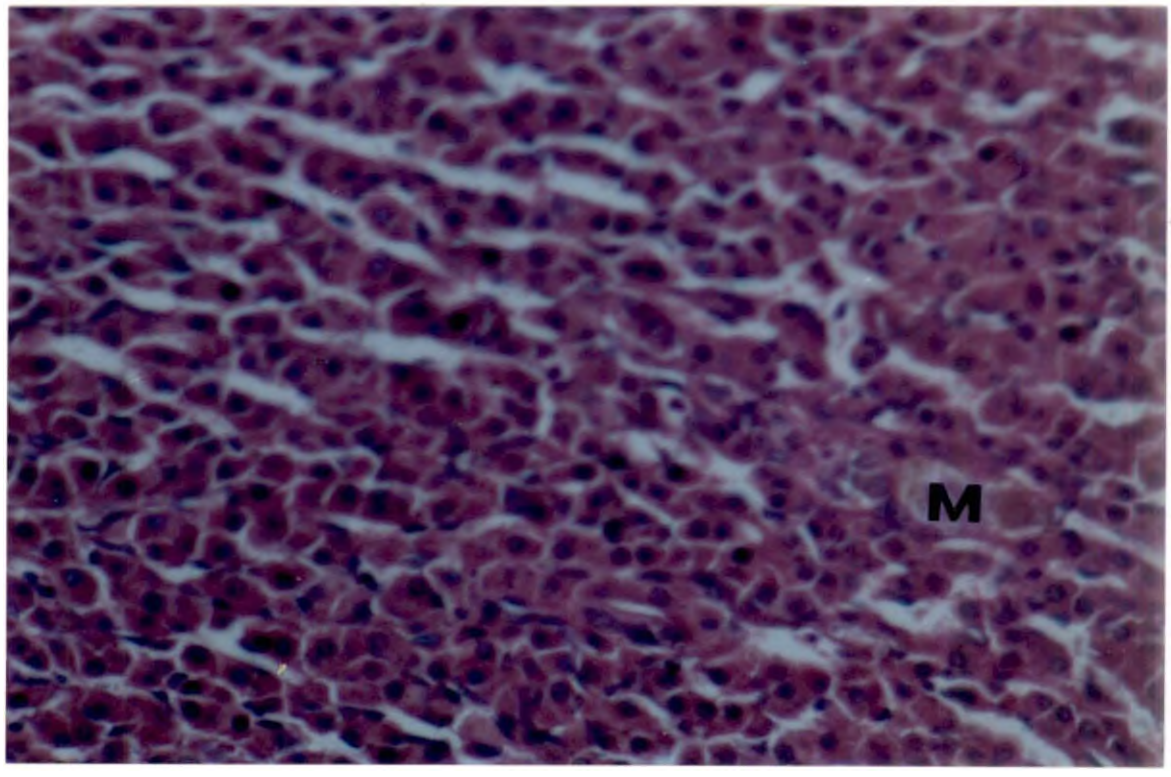

M

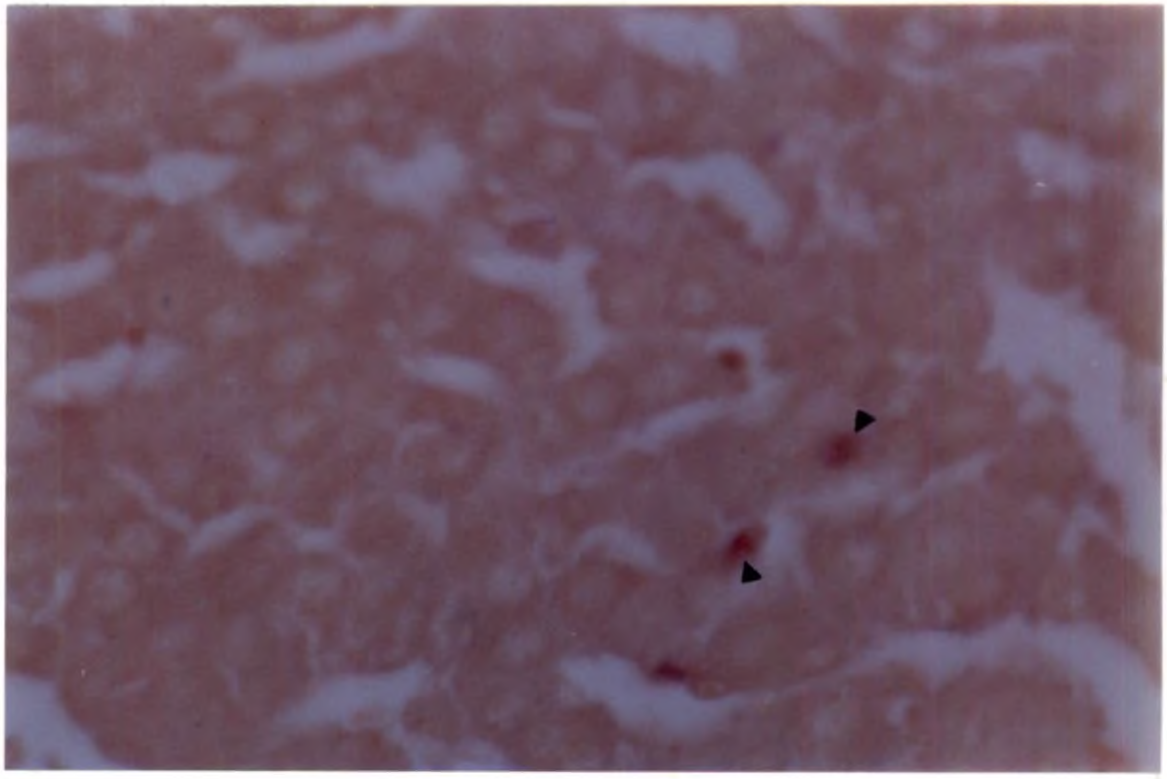
Fig.46 Section of adrenal (150 days - postnatal) showing lipofuscin pigments in the cells of zona reticularis (arrows)

AFIP method x 787.5

Fig.47 Section of adrenal (150 days - postnatal) showing cells of the peripheral and central zones of the medulla

P - Peripheral zone
C - Central zone
S - Sinusoids

H&Ex125



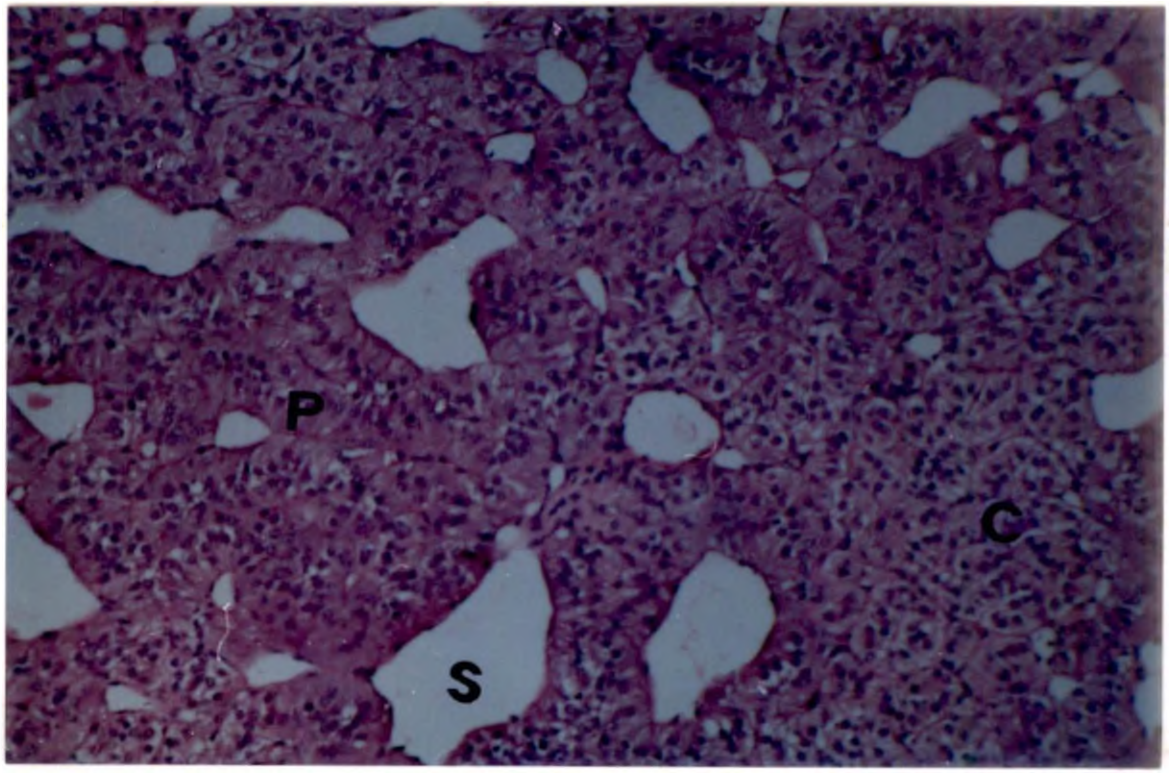

P
C
S

Fig.48 Section of adrenal (90 days - postnatal) showing arrangement of cells in the peripheral (P) and central (C) zones of the medulla

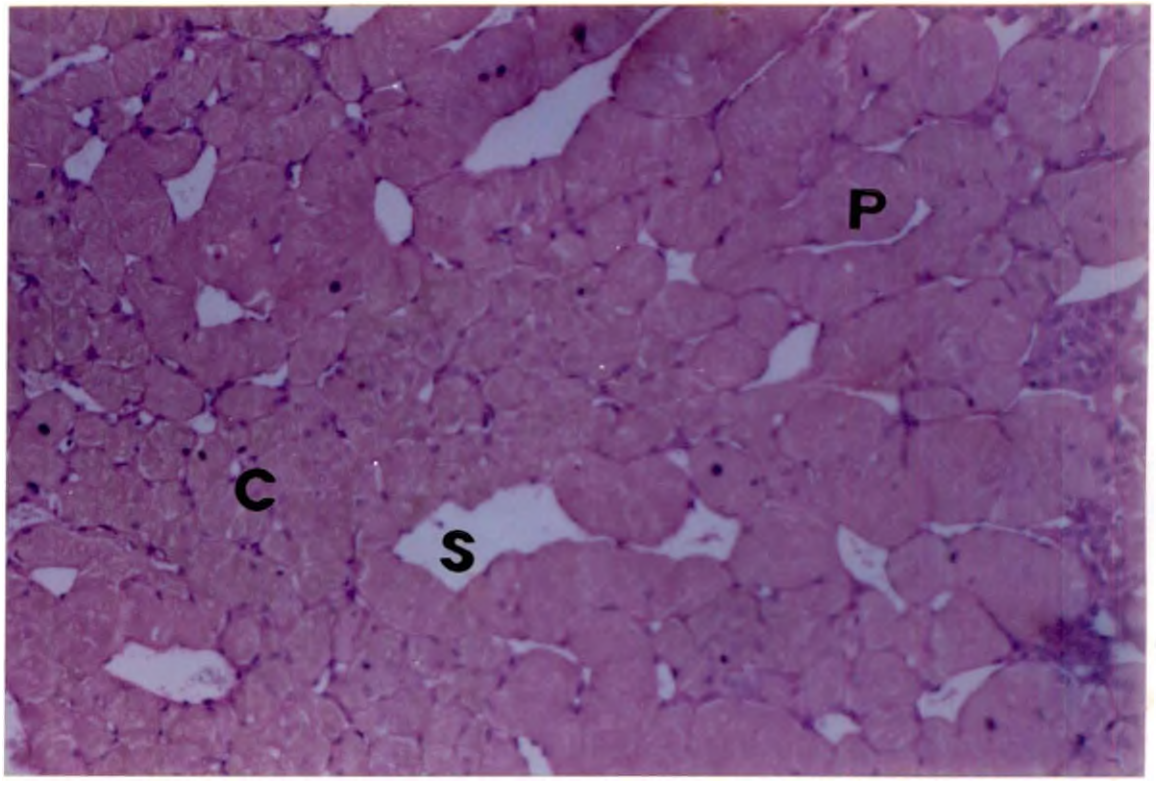
S - Sinusoids

H&Ex125

Fig.49 Section of adrenal (15 days - postnatal) showing epinephrine and norepinephrine cells of the medulla.

CM - Cortical cells in the medulla
CV - Central vein
E - Epinephrine cells
NE - Norepinephrine cells

Wood's technique x 125


P
C
S

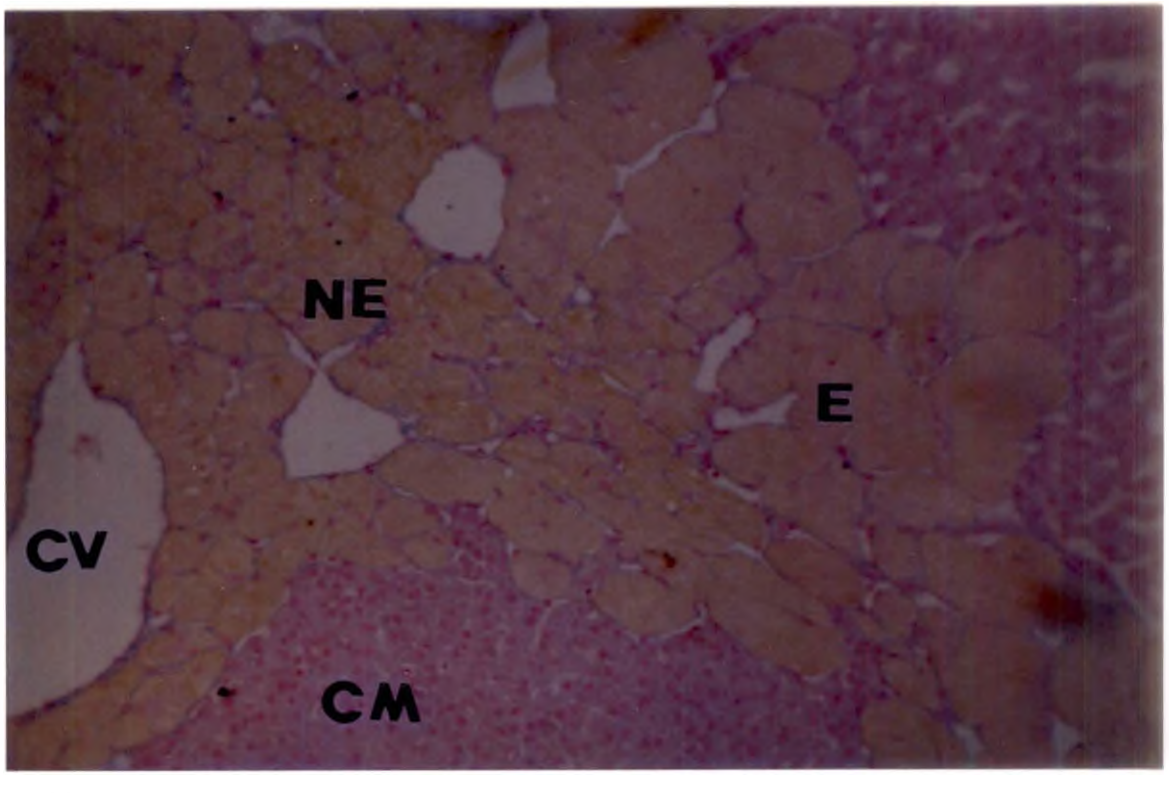

NE
E
CV
CM

Fig.50 **Section of adrenal (42 days foetus) showing moderate PAS positive reaction in the cortical cells. Note strong PAS reaction in the developing metanephric tubules**

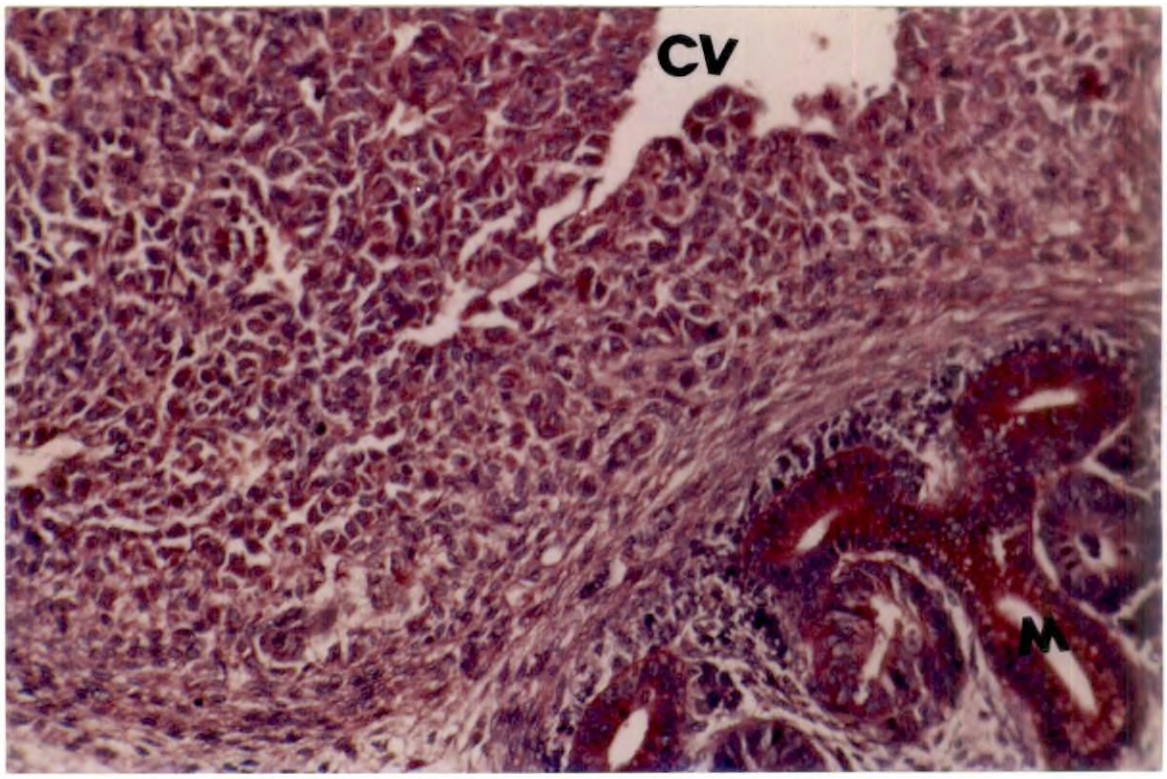
CV - Central vein
M - Metanephric tubule

PAS method x 200

Fig.51 **Section of adrenal (150 days - postnatal) showing weak PAS positive reaction in zona glomerulosa and zona intermedia and moderate reaction in zona fasciculata**

G - Zona glomerulosa
I - Zona intermedia
F - Zona fasciculata

PAS method x 312.5


CV
M

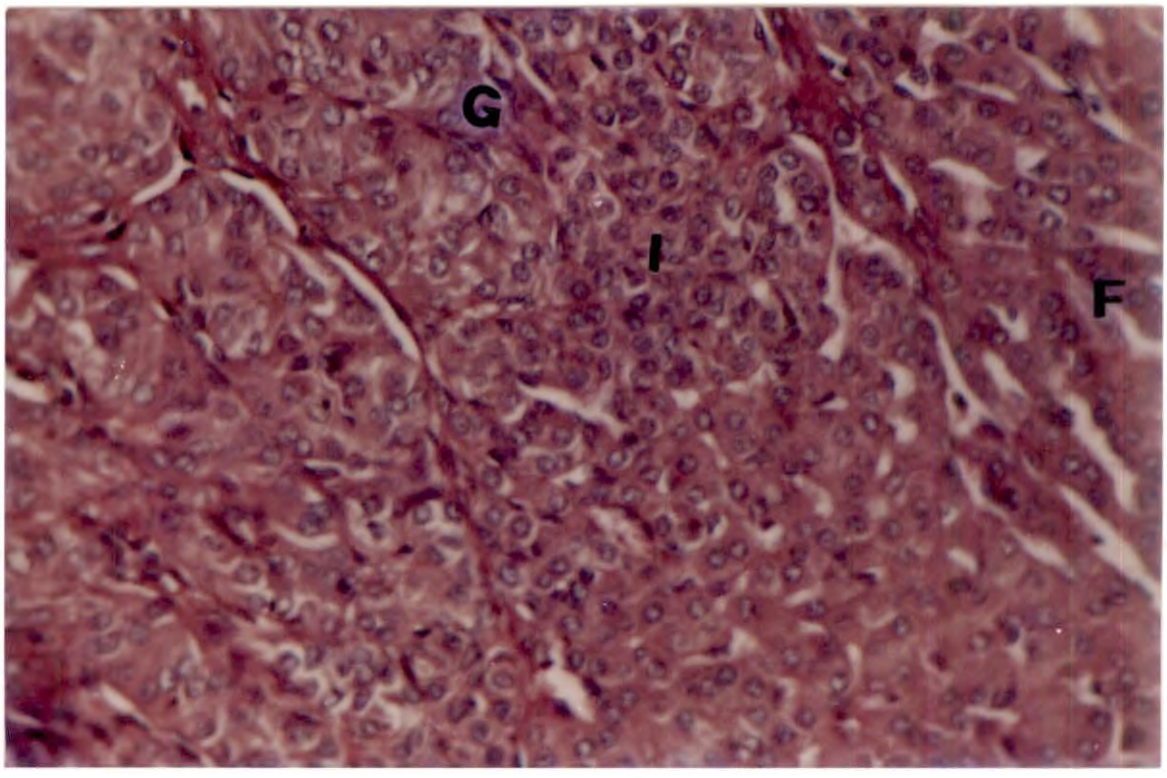
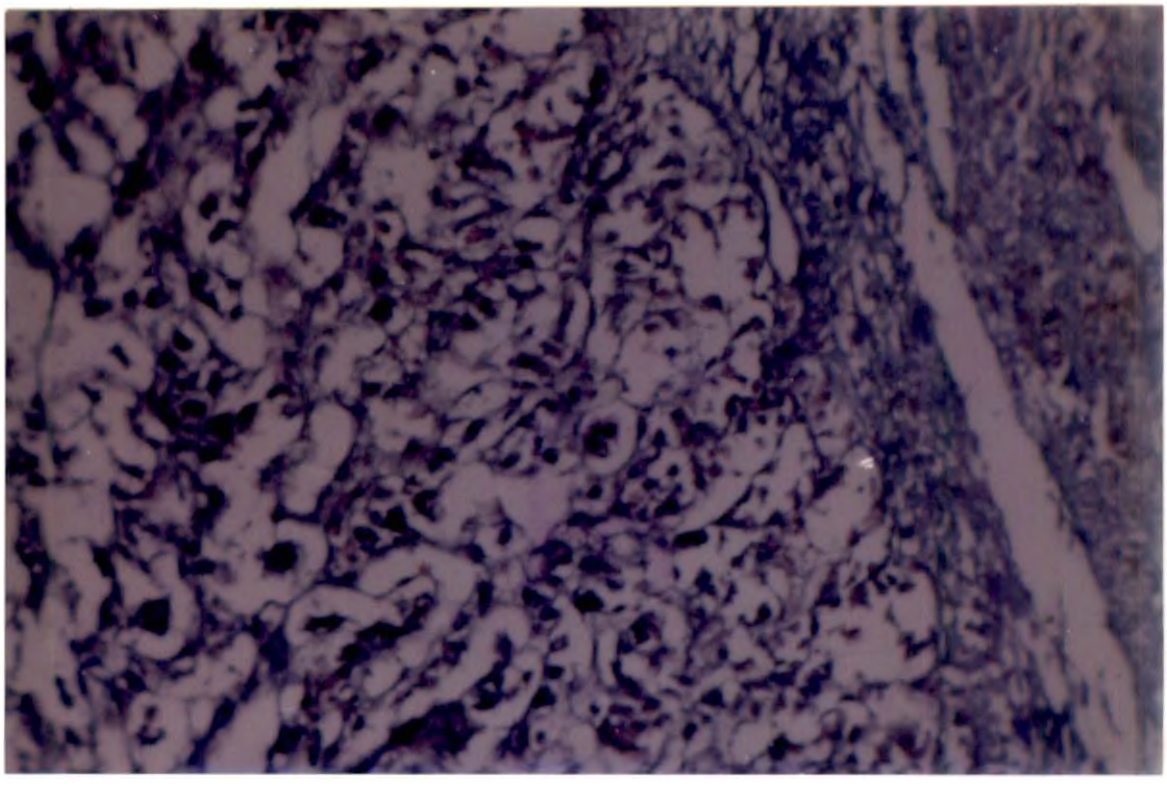

G
I
F

Fig.52 Section of adrenal (73 days foetus) showing moderate reaction for acid mucopolysaccharides in the capsule and weak reaction in the cortical cells

Alcian blue method x 200

Fig.53 Section of adrenal (60 days - postnatal) showing acid mucopolysaccharide reaction in the capsule and cortical zones

Alcian blue method x 125



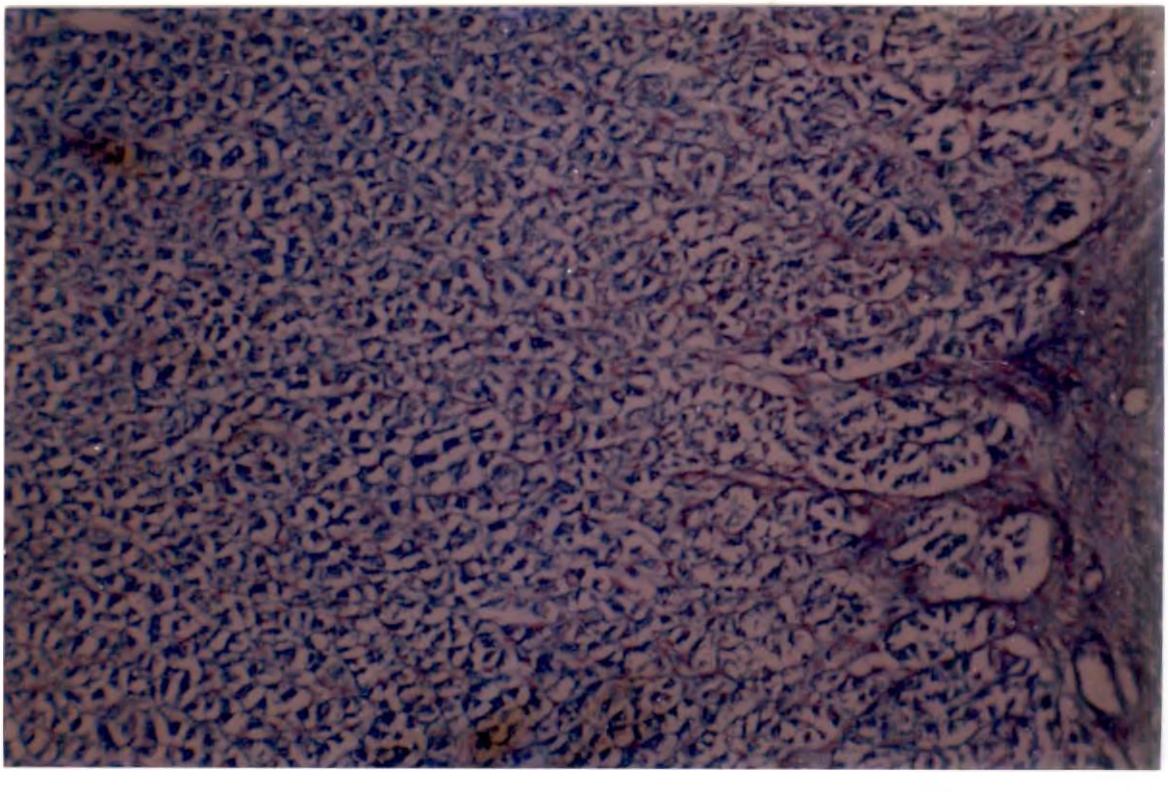


Fig.54 Section of adrenal (60 days - postnatal) showing acid mucopolysaccharide reaction in the zona reticularis and medulla

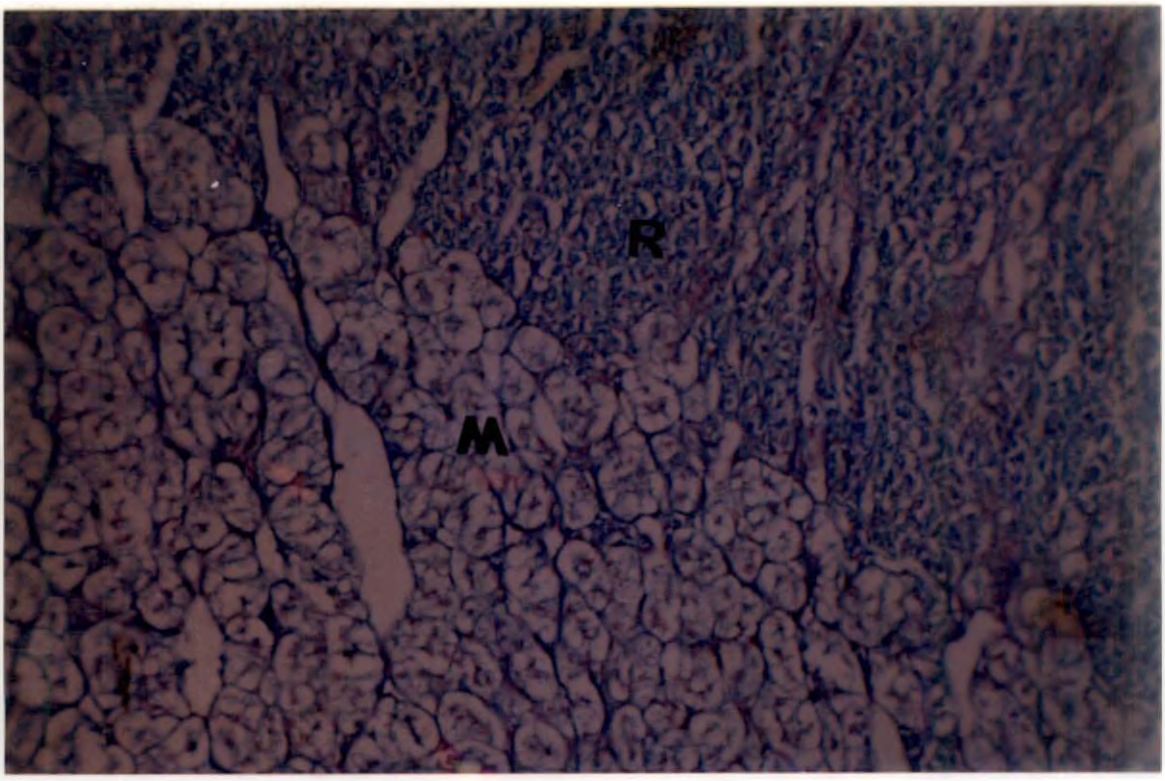
R - Zona reticularis
M - Medulla

Alcian blue method x 125

Fig.55 Section of adrenal (73 days foetus) showing glycogen in the parenchyma

C - Capsule

Best's Carmine method x 312.5


R
M

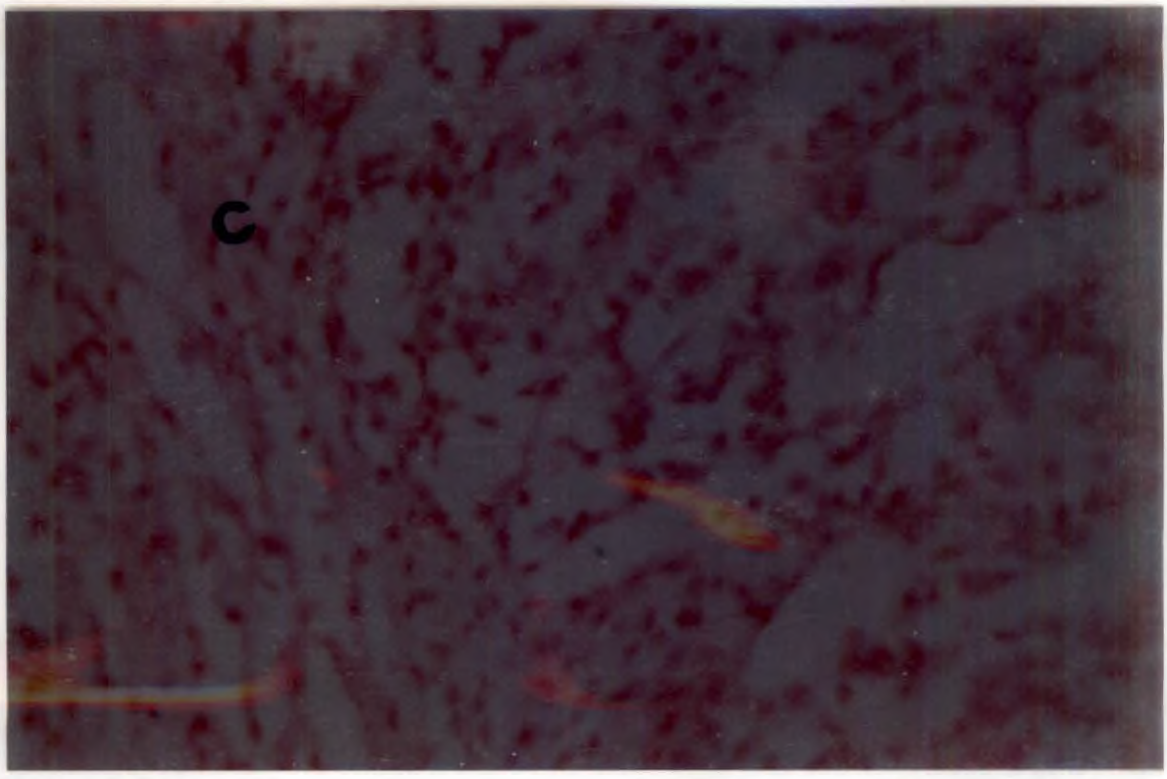

C

Fig.56 Section of adrenal (74 days foetus) showing alkaline phosphatase activity in the cortical cells

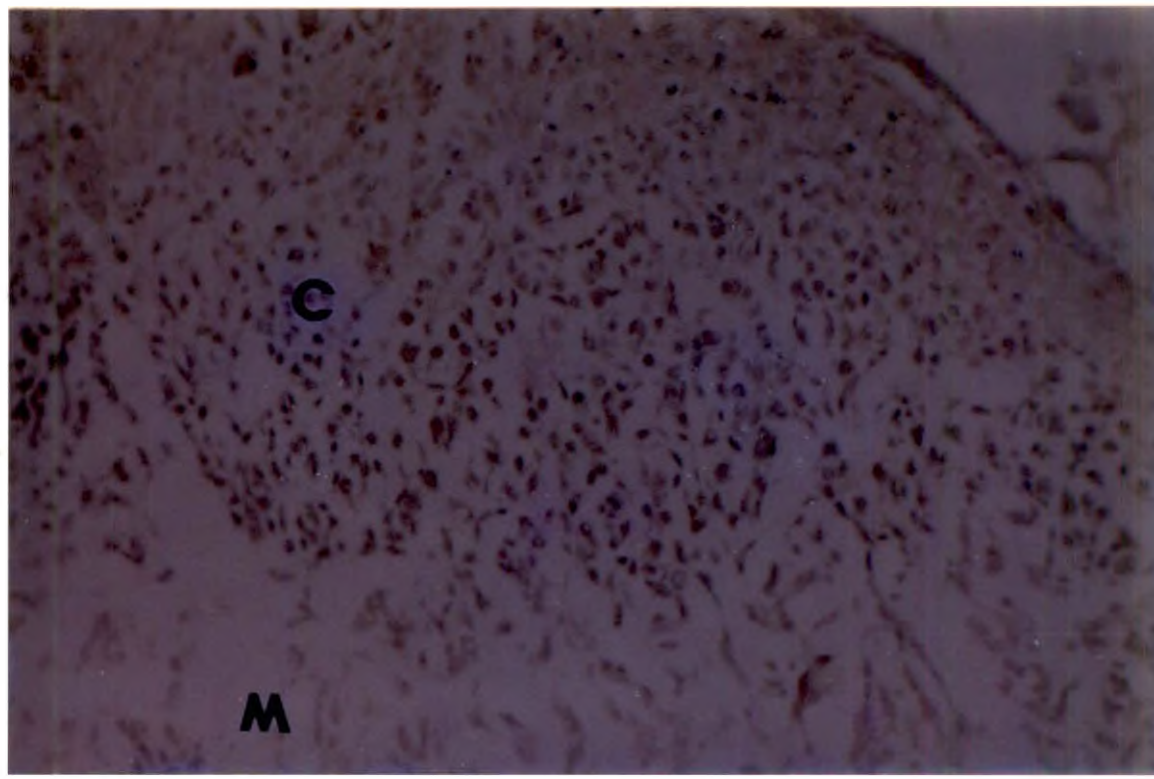
C - Cortex
M - Medulla

Modified Gomori's method x 200

Fig.57 Section of adrenal (30 days - postnatal) showing strong acid phosphatase activity in the zona fasciculata and moderate activity in the zona reticularis

F - Zona fasciculata
R - Zona reticularis
M - Medulla

Naphthol AS-B1 Phosphate method x 200


C
M

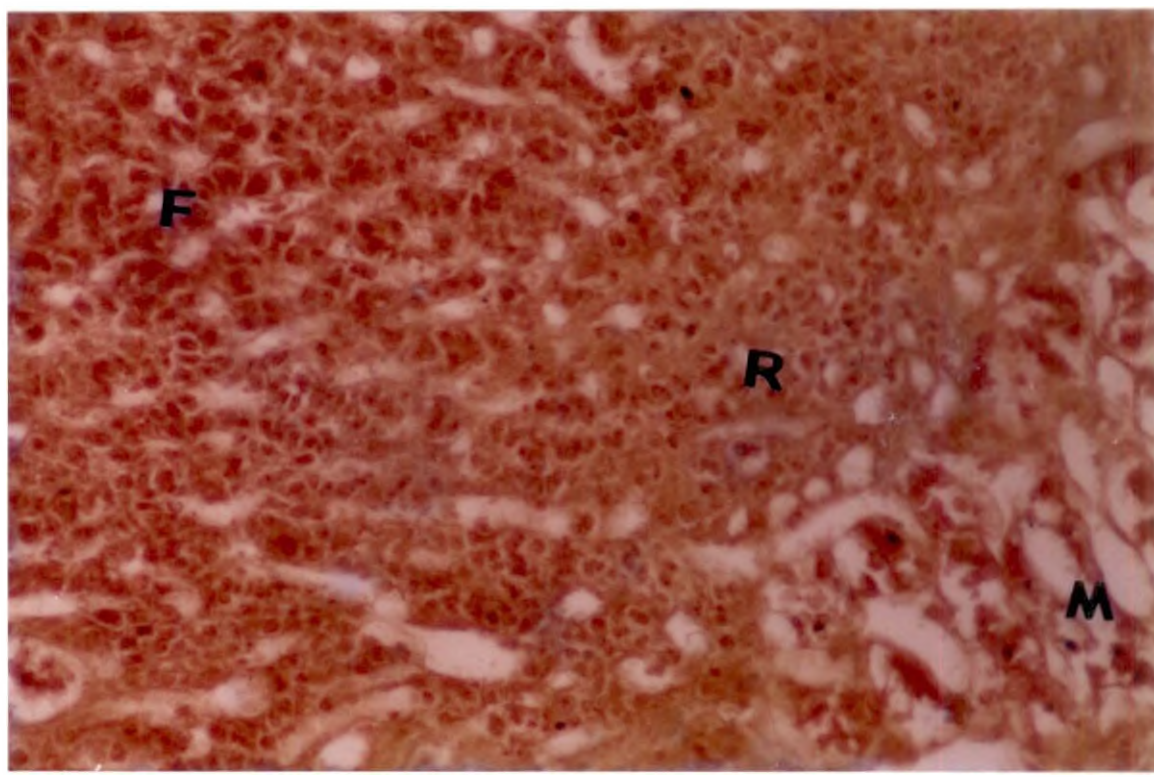

F
R
M

Fig.58 **Section of adrenal (120 days - postnatal) showing strong acid phosphatase activity in the medullary cells**

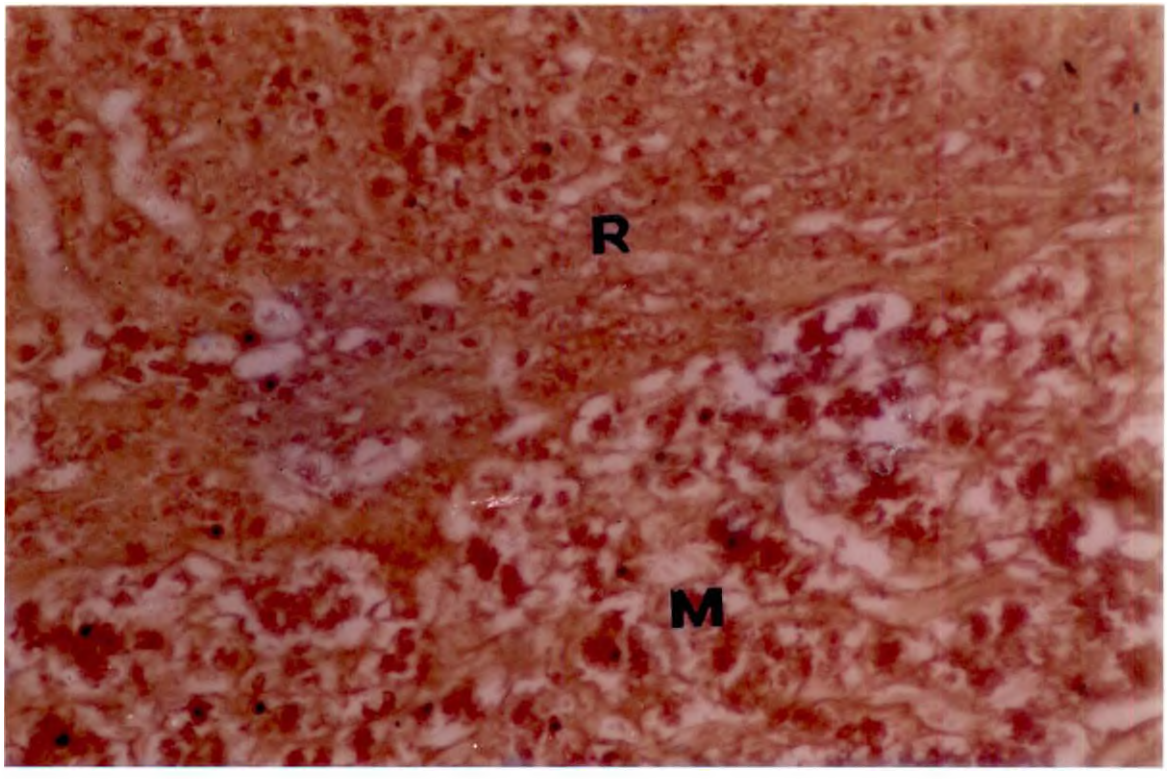
R - Zona reticularis
M - Medulla

Naphthol AS-B1 Phosphate method x 200

Fig.59 **Section of adrenal (30 days - postnatal) showing iodate positive norepinephrine cells towards the central part of medulla**

C - Central zone
P - Peripheral zone
Frozen section

Iodate method x 200


R
M

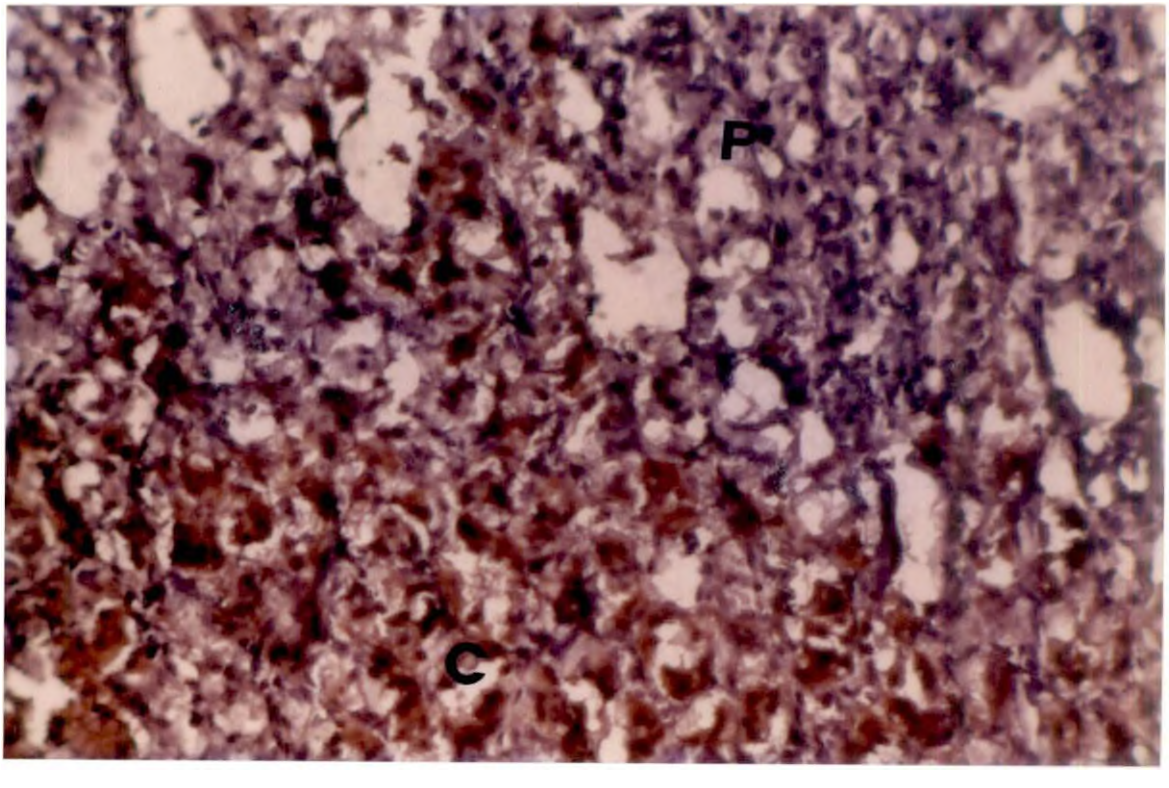

P
C

Fig.60 Section of adrenal (60 days - postnatal) showing lipid droplets in the cortical mass surrounding the central vein of medulla

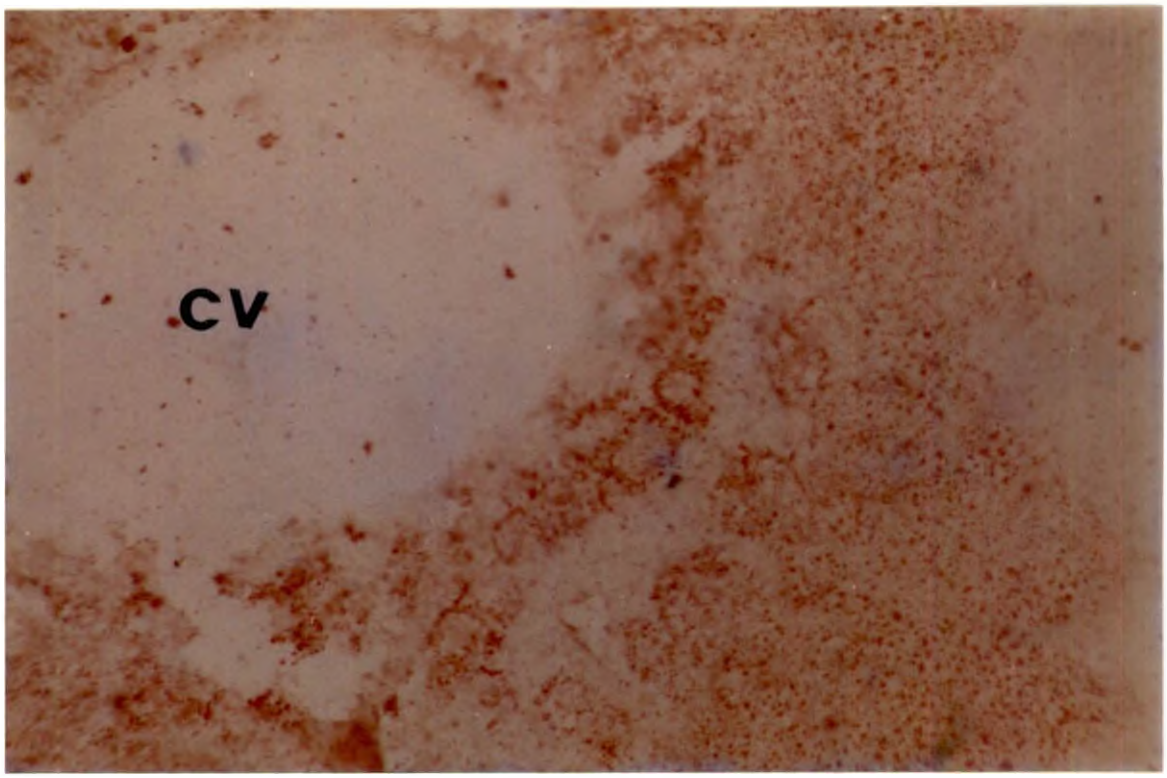
CV - Central vein
Frozen section

Oil Red `0' in propylene glycol method x 125

Fig.61 Section of adrenal (day old kid) showing heavy accumulation of lipid in the cortex

C - Cortex
M - Medulla
Frozen section

Oil Red `0' in propylene glycol method x 125


CV

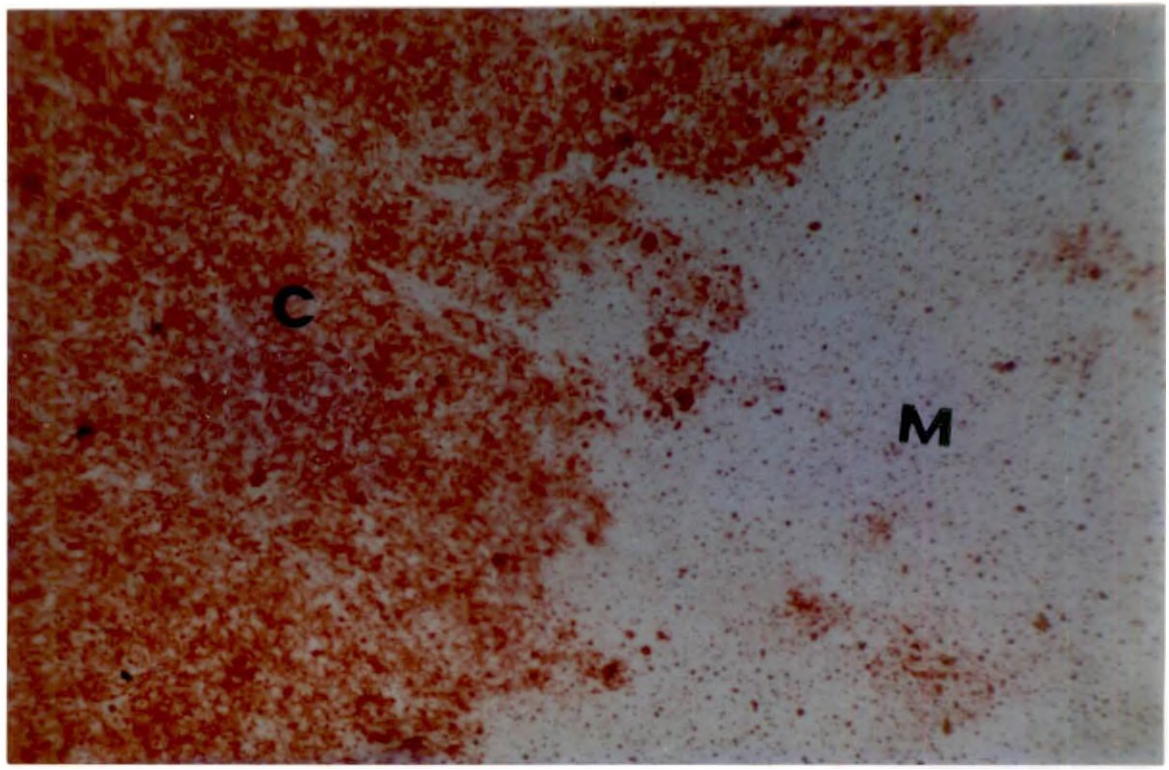

C
M

Fig.62 Section of adrenal (45 days - postnatal) showing heavy accumulation of lipid in the cortex

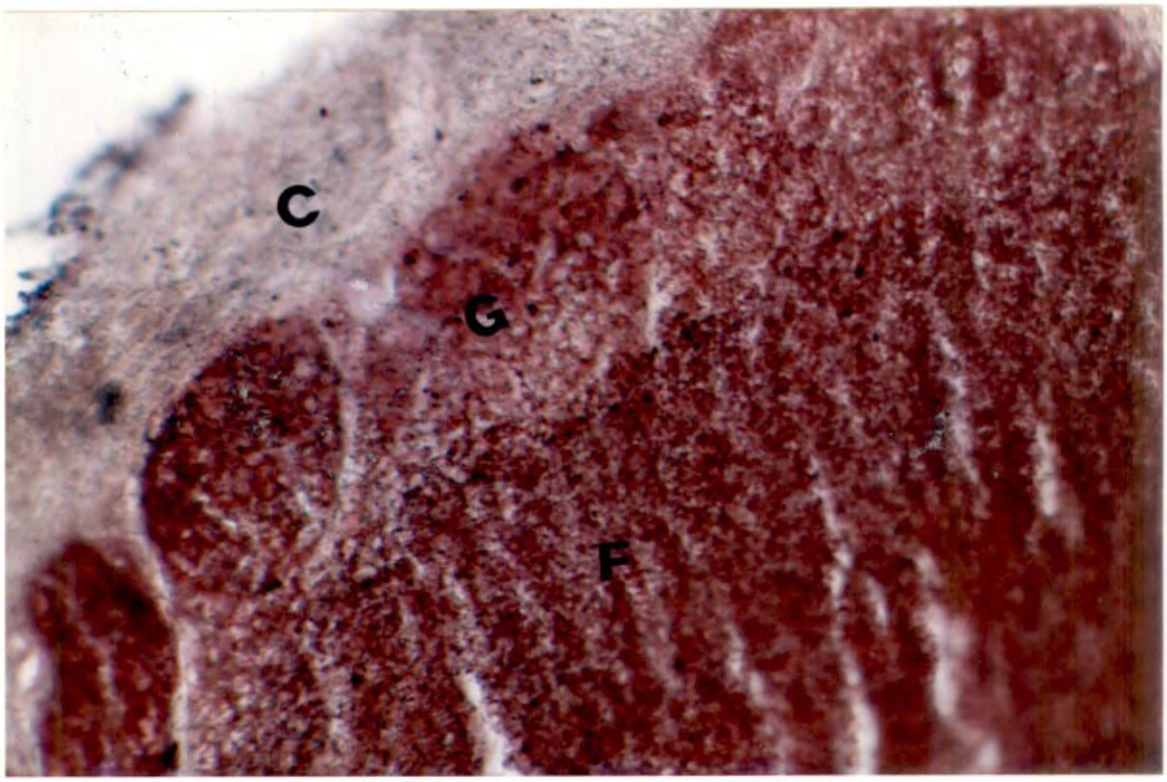
C - Capsule
G - Zona glomerulosa
F - Zona fasciculata
Frozen section

Oil Red `0' in propylene glycol method x 125

Fig.63 Section of adrenal (60 days - postnatal) showing substantial amount of lipid in the zona glomerulosa

C - Capsule
G - Zona glomerulosa
F - Zona fasciculata
Frozen section

Oil Red `0' in propylene glycol method x 125


C
G
F

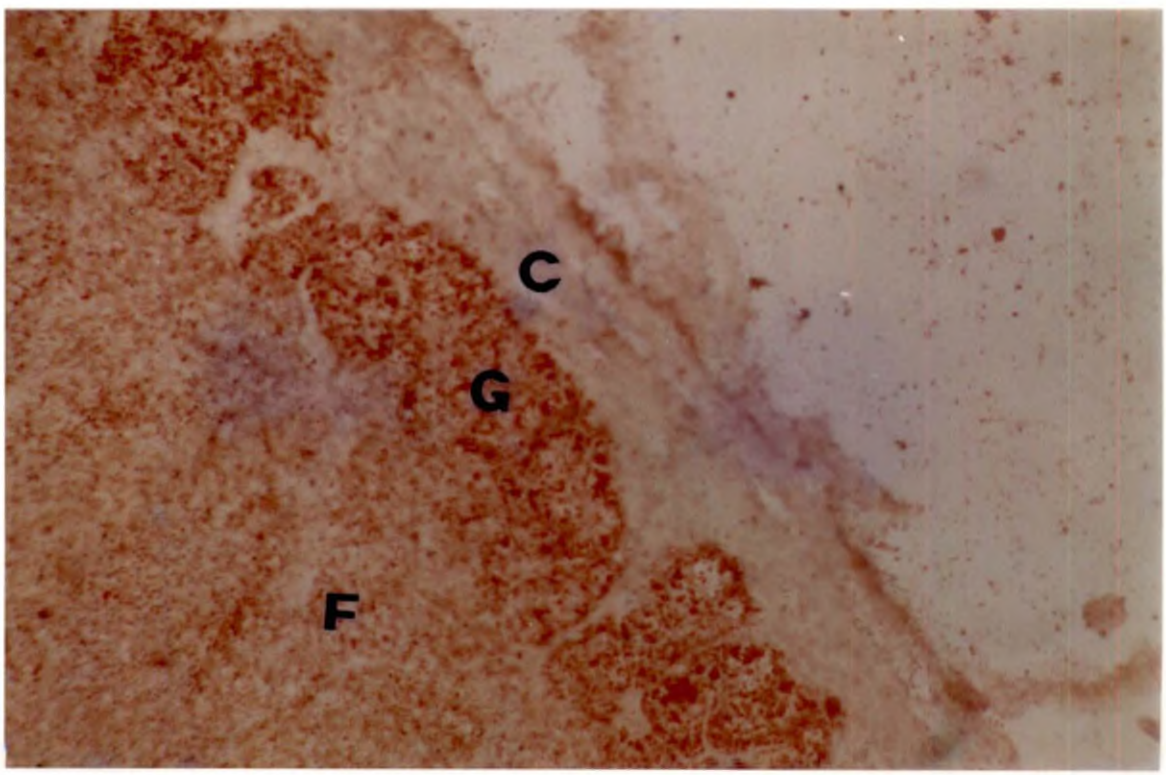

C
G
F

Fig. 64 Relationships between the age and thickness of capsule, width of cortical zones and diameter of medulla in prenatal group

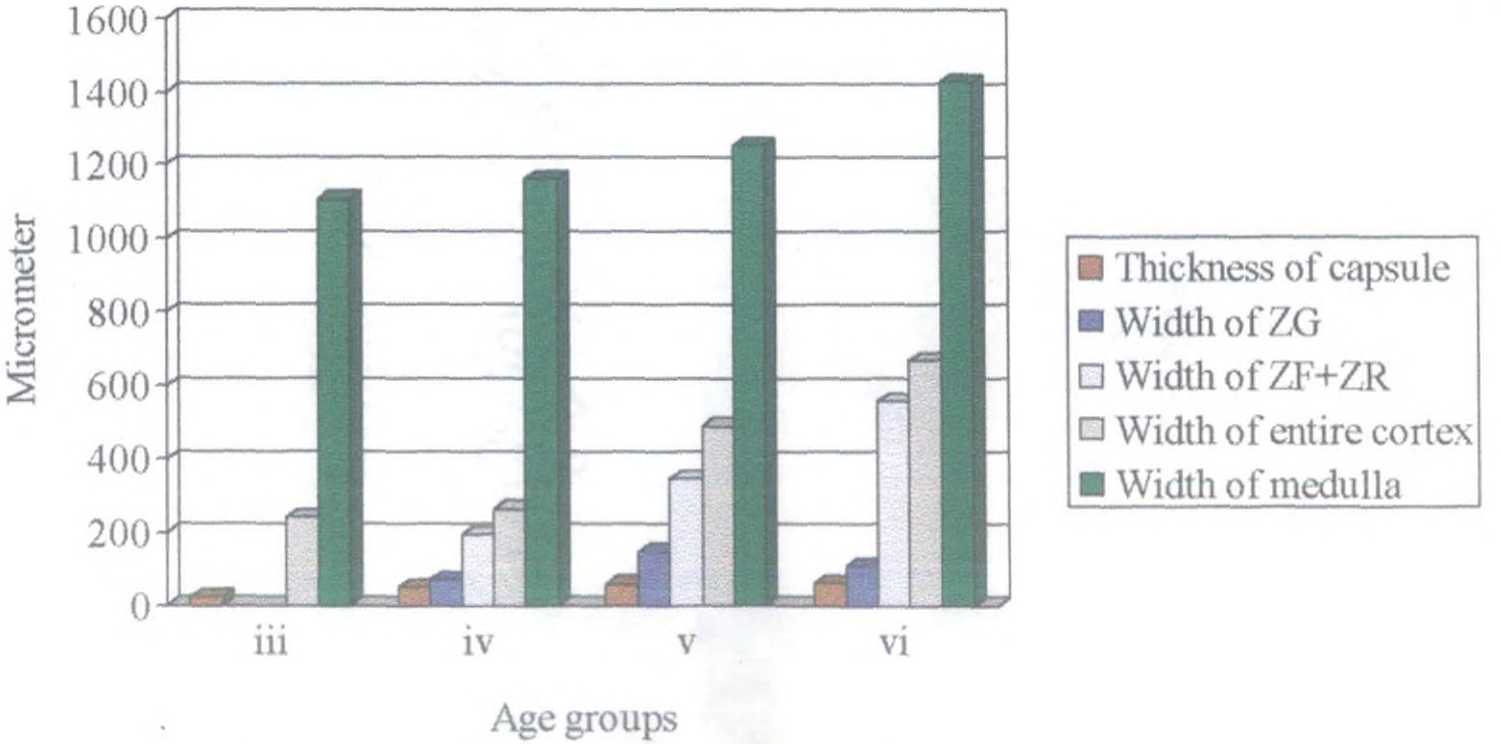


Fig. 65 Relationships between the age and thickness of capsule, width of cortical zones and diameter of medulla in postnatal group

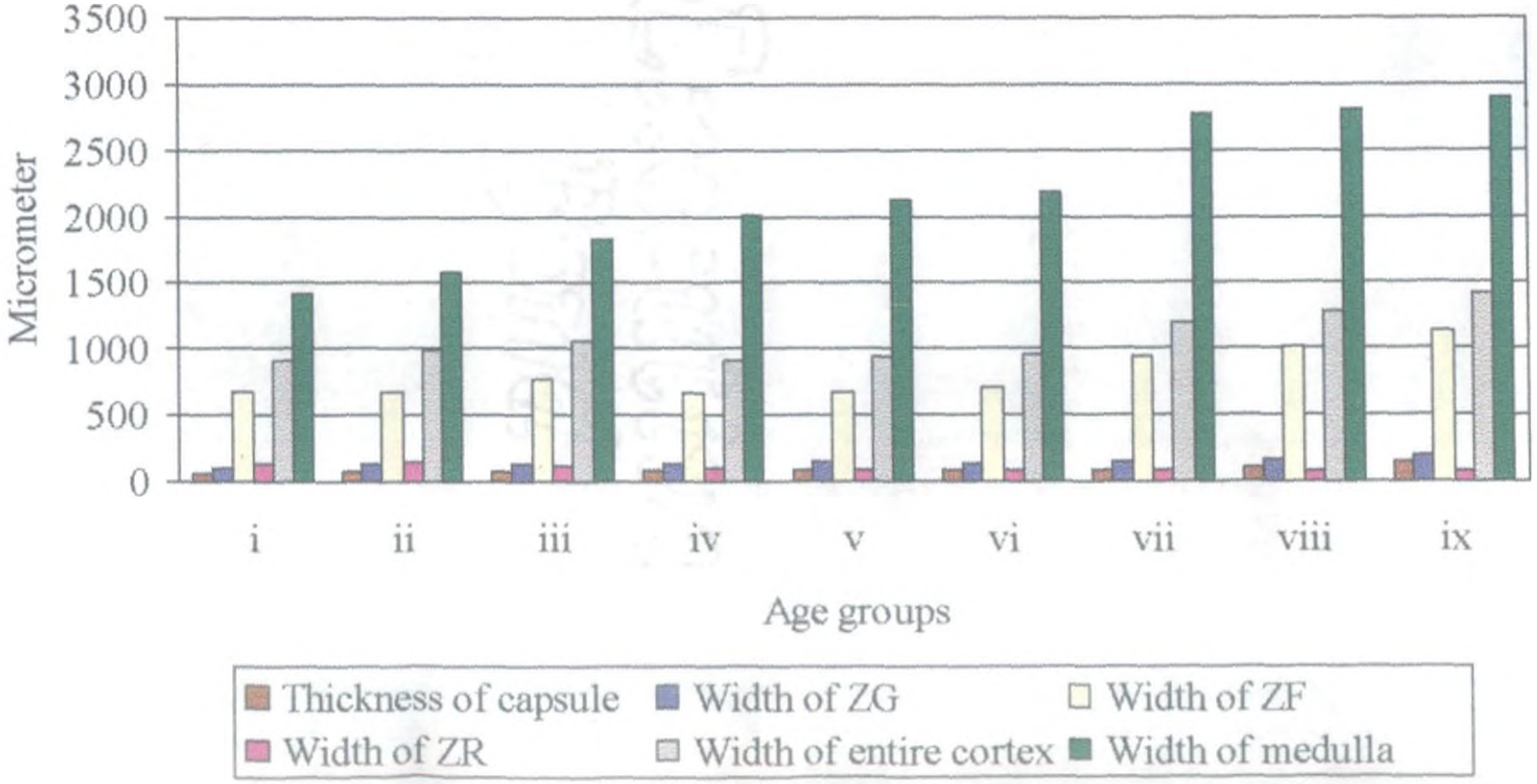


Fig. 66 Relationships between the age and zona glomerulosa/ rest of the cortex ratio and cortex / medulla ratio in prenatal group

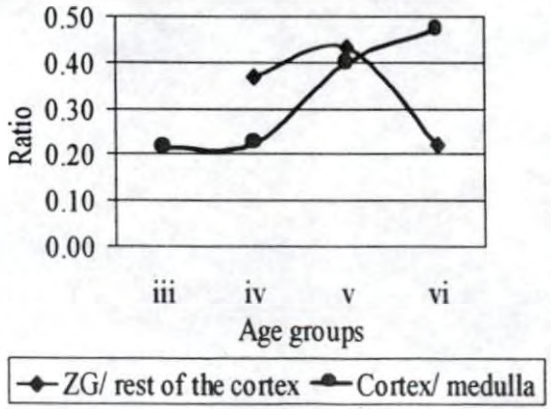


Fig. 67 Relationships between the age and zona glomerulosa / zona fasciculata ratio and cortex/ medulla ratio in postnatal group

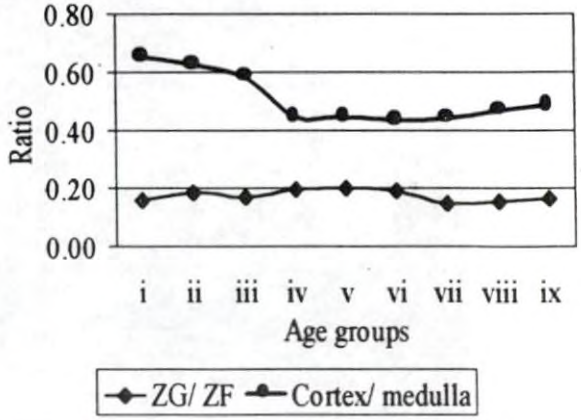


Fig. 68 Relationships between combined weight of adrenals and width of cortical zones in prenatal group

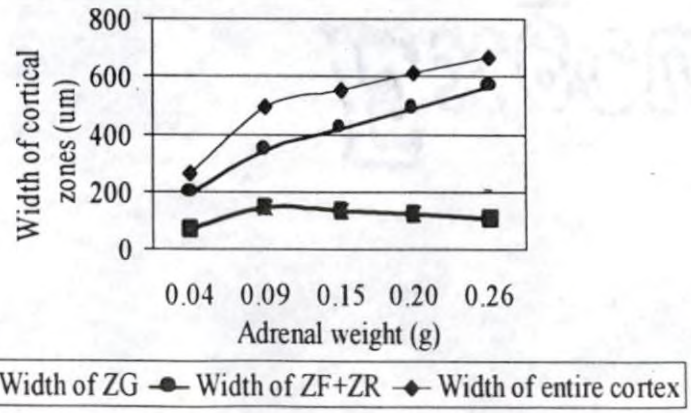


Fig. 69 Relationships between combined weight of adrenals and width of cortical zones in postnatal group

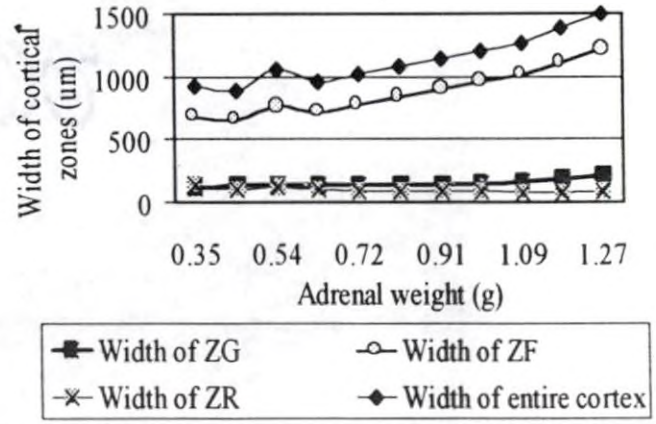


Fig. 70 Percentage contribution of different cortical zones in prenatal group

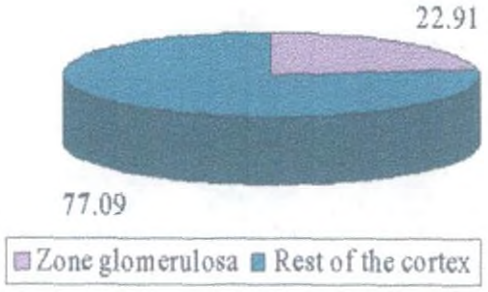
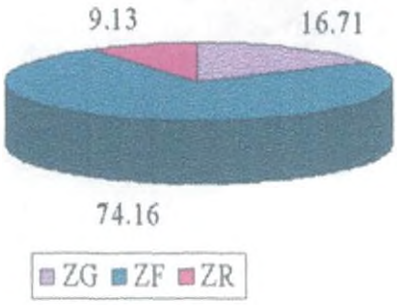


Fig. 71 Percentage contribution of different cortical zones in postnatal group



Discussion

DISCUSSION

In goats the adrenals were located in the retroperitoneum as seen in other species. Glands of both sides were identifiable from their characteristic shapes. The right one was roughly triangular while the left one was slightly elongated. The shape of right adrenal was somewhat similar to that of ox and the left one showed resemblance to that of sheep as reported by Venzke (1975) in these species. From the early foetal age itself, both glands assumed these characteristic shapes.

Weight of the adrenals increased steadily upto 141 days of foetal age. There after a sudden spurt in the weight was recorded until term. A steady increase in the foetal adrenal weight during pregnancy, especially towards the end of gestation has been reported in man (Soffer *et al.*, 1961; Eberlein, 1971; Jost, 1975; Biggs and Klopper, 1977). The sharp increase in adrenal weight from 141 days to term noticed here concurs with the findings in sheep (Comline and Silver, 1961; Thurley, 1972; Jost, 1975). Boshier *et al.* (1980) and Boshier and Holloway (1989) reported that the adrenal weight doubled from 136 days to birth in sheep foetuses. A similar trend was reported in equine (Yamauchi, 1979) and swine (Lohse and First, 1981) foetuses also.

In the present study, the combined adrenal weight showed a high positive correlation with age and body weight of the foetuses. Tiniakos *et al.* (1979) found that in porcine foetuses also the adrenal weight increased with the body weight. In West African dwarf goats, Osuagwuh and Aire (1992) observed positive relationship between adrenal weight and gestational age. They opined that the growth pattern of the gland was related to their physiological role during pregnancy. However, the percentage of the adrenal weight to the body weight showed a decreasing trend in foetuses with a negative correlation with the age. Jost (1975) reported an increase in the relative combined adrenal weight to the body weight between three and four months followed by a decrease until birth in human foetus. He further observed that during the first part of development, the adrenals grew more rapidly than the whole body in many species including the sheep. However, in the present study no such trend was recorded. The percentage weight of the combined adrenals to the body weight showed a steady decreasing trend.

Lohse and First (1981) and Arthur *et al.* (1982) noticed a fast increase in the adrenal growth compared to the foetal body growth during the last week of gestation in domestic animals which was reported to be due to hypertrophy and hyperplasia of the zona fasciculata. This was reflected by a high adrenal to body weight ratio in the last 10 days of

gestation which was peak at birth. In the present study also a similar trend was observed due to the hypertrophy and hyperplasia of the cortex, particularly of the zona fasciculata. But the adrenal to body weight percentage did not increase significantly as reported by Lohse and First (1981) in foetal pigs. This could probably be due to a concommitant and proportionate increase in the foetal body weight along with the adrenal weight increase.

It has been proved that towards the end of gestation placenta is not fully capable of meeting the increased demands of the foetus. This induces stress on the foetus, thereby increasing the foetal cortisol output via. the foetal ACTH (Arthur *et al.*, 1982). Nussdorfer *et al.* (1978) observed that stress caused an increased ACTH output which in turn caused increased adrenal weight and steroidogenic capacity of the cortical cells. A spurt in the adrenal weight after 141 days of gestation noticed in the present study could be due to the effect of enhanced foetal ACTH production resulting from the increased stress on the growing foetus.

Comline and Silver (1961) also noticed rapid growth of adrenal and a mature histological picture in sheep foetuses towards the end of gestation. If has been proved that the maturation of the foetus and parturition are closely linked together. These two are related to the adrenal cortical function and the prepartum surge of foetal cortisol (Liggins,

1976; Reperant and Durand, 1997). An increased corticosteroid secretion from the foetal adrenal gland towards term and after birth has been reported in man (Jones *et al.*, 1977; Murphy, 1982), sheep (Comline and Silver, 1961; Alexander *et al.*, 1968; Basset and Thorburn, 1969; Rees *et al.*, 1975) and pigs (Lohse and First, 1981; Randall, 1983). Challis and Currie (1983), Brieu *et al.* (1988) and Fowden and Silver (1988) found that this increased corticosteroid secretion from the foetal adrenal was due to a higher production of ACTH from the foetal pituitary. The changes in the foetal adrenal gland observed in the present study support the above findings.

After birth also the adrenal weight showed an increasing trend upto 180 days with a reduction at 30 and 45 days. At parturition exposure of the young one to the atmosphere, induces stress and this could be responsible for the increased weight of the glands during early neonatal periods. Once the young one gets acclimatized to the external environment, effects of stress are removed and thus the adrenal weight again gets reduced by about 30-45 days postpartum.

The combined adrenal weight was positively correlated with age and body weight during postnatal period also. However, the proportion of adrenal weight to body weight percentage showed a decreasing trend. This indicates that after birth also, the adrenal growth rate was not in proportion to the body growth rate. The whole body grew at a

faster rate compared to the adrenals. An observation similar to this was made in pigs by Tiniakos *et al.* (1979).

The left adrenal was slightly heavier than right during the prenatal and the postnatal periods. In cattle (Venzke, 1975), sheep (Thwaites and Edey, 1970) and pigs (Warriss, 1981) also similar findings have been reported.

The length, breadth and thickness of both the glands showed an increasing trend with the advancement of age, before and after birth. However a marginal reduction at 45 days postpartum was also observed. This could be attributed to a decreased activity of the gland at this stage when the neonatal stress was relieved. All these parameters were positively correlated with the age in both the groups studied. Left adrenal was found to be longer than right while the breadth and thickness were more for the right gland throughout the period of study. However in buffaloes, Nagra *et al.* (1989) could not find any such difference between the two glands. According to Venzke (1975), the left gland was slightly broader and thicker than the right, but no difference was reported between the length of the glands in cattle.

The developing adrenals had direct anatomical continuity with the mesonephros and the developing gonad in the present study. Upadhyay and Zamboni (1982) recorded similar results

in the sheep. They opined that the mesonephros was the primary source of the adrenal cortex in this species.

Adrenal anlagen was first detected in 33 days old embryos as whorls of cells. This was seen cranial to developing metanephros and dorsomedial part of the mesonephros along the ventrolateral aspect of aorta. These findings agree with those reported in other domestic ruminants (Venzke, 1975). Upadhyay and Zamboni (1982) detected cortical primordium at 28 days of development in sheep. Robinson *et al.* (1979) identified the adrenal anlagen at 30 days old sheep foetuses. In rats, Lever (1955) observed the adrenal primordium in 12-13 days of foetal life whereas, El-Maghraby and Lever (1980) noticed the primordium at 16 days of gestation only. Hager (1965) noticed the beginning of cortical formation between 28 and 33 days in bovine foetus. In pig embryos, Bielanska-Osuchowska (1989a; 1989b) detected the cortical primordia as early as 21 days of gestation.

No `foetal cortical cells' were detected in the developing adrenal in the present material as reported in man and monkeys by Uotila (1940), Lanman (1953), Soffer *et al.* (1961), Benirschke and Richart (1964) and Jost (1975).

By 36 days, these primordial cells gradually organized into cords and dense aggregations. The cells possessed deep eosinophilic cytoplasm with oval or spherical nuclei. This

concurs with the findings of Robinson *et al.* (1979) in sheep foetuses.

A thin capsule around the primordium composed of collagen fibres was detected first by 42nd day of gestation. Very thin collagen fibres were noticed among the parenchymal cells also at this stage. Division of the capsule into outer more fibrous and inner more cellular layers was apparent by 70 days. Hakeem *et al.* (1993) also detected thin fibroelastic capsule and trabeculae during early foetal life in goats. In sheep foetuses, the adrenal capsule became apparent by 40 days (Robinson *et al.*, 1979). In guinea pigs, a distinct capsule was noticed by 27 days of foetal age (Black, 1972). In the present study reticular fibres were recorded in the capsule by 58 days and the elastic fibres by 74 days. However, Hakeem *et al.* (1993) located reticular fibres in the later part of gestation only, in goats.

Histological differentiation of the gland started by 42 days. The cells were organized into small groups and cords separated by irregular spaces. The first sign of central vein formation was noted at this age. These tally with the findings of Robinson *et al.* (1979) in sheep foetuses.

Between 46 and 75 days of development, size of the glands increased. Anatomical continuity with the degenerating mesonephros and the developing gonad was not visible after 50

days. The glands could be clearly demarcated from the surrounding structures. Bielanska-Osuchowska (1989b) recognized the gland as a separate organ with a capsule at around 50 days of foetal age in pigs. However, Upadhyay and Zamboni (1982) reported that the anatomical continuity had lost as early as 31 days of gestation in sheep.

The future medullary cells started migration to the cortical primordium by 50 days of development. These cells were seen both within and around the cortical primordium as aggregations or solitary cells. They were smaller than the cortical cells and possessed dark, oval nuclei. The rate of migration of these neural crest cells to the developing gland diminished with advancing foetal age. After 70 days no migration took place. In bovines, Hager (1965) noted penetration of neural crest cells into the cortex by about 50 days of intrauterine life and the appearance of epinephrine and norepinephrine cells at 120 days of gestation. Bielanska-Osuchowska (1989c) also reported a fall in the rate of migration of the neural crest cells with the advancement of gestation in pigs. Such cells were confirmed as neuroblasts that migrated from the nearby sympathetic ganglia by Banks (1981); Ehrlich *et al.* (1989) and Boshier *et al.* (1989). These cells were reported to penetrate the medial aspect of the cortical primordium and transformed into chromaffinoblasts or sympathochromaffin cells.

According to Bielanska-Osuchowska (1989c), the fate of these migratory neural crest cells was determined by the presence or absence of a nerve growth factor (NGF). Presence of NGF caused differentiation of these cells into neurons. In the absence of NGF, under the influence of corticosteroids, they differentiated into chromaffin cells. Seidl and Unsicker (1991) also suggested that glucocorticoids were essential for triggering the differentiation of the neural crest cells into chromaffin cells.

Histological appearance of the migratory cells and the pattern of migration observed in the present study agree with the earlier reports in sheep (Robinson *et al.*, 1979) and pigs (Bielanska-Osuchowska, 1989a, 1989c).

Differentiation of chromaffinoblasts to chromaffinocytes was reported to occur about 10 days prior to farrowing in pigs (Bielanska-Osuchowska, 1989c). But in the present study norepinephrine cells were present from 50 days of foetal life itself. However, epinephrine cells made their presence only by 98 days of development. Their number showed a steady increase towards term. According to Comline and Silver (1961) the percentage of adrenaline increased with age in foetal sheep. It has been proved beyond doubt that epinephrine together with ACTH and cortisol stimulate lung maturation, thus enabling normal respiratory function to occur in new born (Arthur *et al.*, 1982). Cells with typical characters of

multipolar neurons were detected by 62 days onwards in the present study. In man, Eberlein (1971) recorded invasion of sympathocytoblasts from 44 days onwards. Soffer *et al.* (1961) opined that their further differentiation occurred only at a later stage. In rats, invasion of sympathochromaffin cells started by 16th day and continued upto the late prenatal period (Lever, 1955). Seidl and Unsicker (1991) noted the differentiation of such cells began between 16 and 17 days of gestation and epinephrine was detected after 17 days only.

Boshier *et al.* (1989) observed two types of migratory neural crest cells in the sheep foetuses. Contrary to this the present material showed only one cell type.

The parenchymal cells just beneath the capsule began to organize into small clusters by 58 days of development. By 95 days the zona glomerulosa was well distinguishable. In rats, Idelman (1970) observed the differentiation of zona glomerulosa by 18 days and completion by 20 days of foetal life. In the bovine, this process was reported to begin at about 50 days of foetal life (Hager, 1965). According to *Robinson et al.* (1979), eventhough the histological differentiation of the adrenal started from 40 to 60 days in sheep foetuses, differentiation of zona glomerulosa began only after 60 days. After 80 days this zone attained the appearance of mature zona glomerulosa. In guinea pigs (Black, 1972) and rabbits (Albano *et al.*, 1976), the differentiation

started at 27 and 24 days respectively. In equines, Yamauchi (1979) recorded both the zona glomerulosa and the zona fasciculata by 4th month of pregnancy. According to Lohse and First (1981), in foetal pig the adrenocortical zonation began by day 89 and was completed by day 113 of gestation. However, Gutte *et al.* (1986) observed signs of glomerular and fascicular zone development by day 80 and reticular zone only after birth in pigs. Hullinger *et al.* (1978) noticed well defined cortical zonation in dogs only in the postnatal period.

Three generations of cortical cells with distinct cellular characteristics were reported by Bielanska-Osuchowska (1989a; 1989b) in foetal pigs. Such cell types were not seen in the present material.

In the present study, the cellular cords towards the centre of the gland were loosely arranged during the early stages of gestation. As the foetal age advanced, they began to organize into tightly packed cords and clusters. By 70 days the corticomedullary separation started which became very distinct by 95 days of development. However interdigitations at the corticomedullary junction were seen throughout the study period. Boshier *et al.* (1989) reported a distinct corticomedullary separation by 100 days of development in sheep foetuses.

The zona fasciculata differentiation started by 98 days of foetal age and by 122 days it became distinguishable. However the zona reticularis was evident by 129 days. By this time all the cortical zones were well differentiated. But Alexander *et al.* (1968) reported that the zona reticularis in sheep differentiated only after birth, though the other two zones were distinguishable in the late foetal period. However, Boshier *et al.* (1980) reported the presence of a zona reticularis in full term sheep foetuses.

Disintegration of cellular cords in some parts of the cortex in foetal sheep was observed by Robinson *et al.* (1979). Such changes were not seen in this study. However, intermingling of cortical and medullary cells in the medullary zone and the absence of a `foetal cortex' observed in this study are in total agreement with the findings of Robinson *et al.* (1979) in sheep foetuses.

Pronounced growth of adrenal cortex particularly of the zona fasciculata after 141 days of gestation observed in the present study confirmed the earlier findings of Durand *et al.* (1978), Liggins *et al.* (1979) and Nicolle and Bosc (1990) in foetal sheep and Hakeem *et al.* (1993) in foetal goats.

The sequence of events taking place in the differentiation and growth of the adrenal cortex are more or less similar to that in various species of domestic animals.

The present observations are in line with the findings of Black (1972) in guinea pigs, Yamauchi (1979) in horses, Boshier and Holloway (1989) in sheep and Hakeem *et al.* (1993) in goats. The differences in time intervals can be attributed to the wide variations in the gestation periods.

Intermingling of cortical and medullary cells was noticed till 104 days of foetal development. As the foetal age advanced, the cortical cells in the medulla gradually disappeared, however groups of cortical cells were seen concentrating near the central vein. These could probably be the cortical cells trapped in the central region of the gland.

Adrenal medulla in full term foetuses revealed numerous follicles containing a colloid material. Similar follicular formations were observed in the adrenal medulla of domestic ruminants by Smollich (1965; 1966). These were reported to be the effect of an acute or extreme functional stress on the medulla. Arthur *et al.* (1982) stated that foetal adrenal medulla showed evidence of maturational changes by producing more catecholamines, especially epinephrine in response to the stress of asphyxia in late gestation.

The general structure of the gland during the postnatal period was similar to that in older foetuses. Studies on sheep adrenal by Alexander *et al.* (1968) revealed similar findings.

The glands were covered by a highly vascular connective tissue capsule composed of collagen, reticular and elastic fibres with a few smooth muscle cells. The capsule was divisible into outer more fibrous and inner more cellular layers. These findings agree with those made in other domestic animals (Trautmann and Febiger, 1957; Prasad and Yadava, 1972; 1974; Dellmann, 1993; Ashok *et al.*, 1994a) including goats (Hakeem *et al.*, 1993). Groups of undifferentiated cells with pale, vesicular nuclei seen in the inner cellular layer of the capsule are similar to those reported by Dellmann (1993) in other domestic animals. These cells were reported to differentiate into the cells of zona glomerulosa.

In addition, cells of the capsule included fibroblasts, multipolar neurons and melanocytes. Islands of fully differentiated cortical cells were also found in the capsule. Existence of melanocytes in the adrenal capsule has already been reported in goats (Jamdar and Ema, 1982a; Hakeem *et al.*, 1993) and in buffaloes (Ashok *et al.*, 1994a). Neurons could be seen either as solitary ones or as components of ganglia within the capsule. Such ganglia might have formed from the migrated neural crest cells that failed to enter the parenchyma. Presence of fully differentiated cortical cells in the capsule is similar to the finding in buffaloes (Ashok *et al.*, 1994a). The supporting framework of the parenchyma

observed in the present study is similar to that found in other domestic animals (Prasad and Yadava, 1972; Dellman, 1993; Hakeem *et al.*, 1993).

Division of the parenchyma into cortex and medulla and further subdivision of the cortex into zona glomerulosa, zona fasciculata and zona reticularis concur with those of other domestic animals (Fahmy *et al.*, 1965; Prasad and Yadava, 1972; Venzke, 1975; Lohse and First, 1981; Banks, 1981; Dellmann, 1993).

Mast cells were recorded occasionally in all the three cortical zones. Hinson *et al.* (1989) recorded mast cells in the walls of the arterioles in the adrenal capsule in rats and suggested that they modulate both vascular and secretory responses in the intact gland.

Presence of cortical tissue in the medulla and medullary tissue in the cortex as islands and podia has already been reported in several other species also (Otsuka, 1962; Prasad and Sinha, 1981a; 1984; Abdalla and Ali, 1988-89; Ashok *et al.*, 1994a; Pignatelli *et al.*, 1995). Payet *et al.* (1987) suggested paracrine function of these medullary cells, via. their secretory products within the cortex.

The cortical cells in the medulla were located mainly around the central vein. Whenever, the trabeculae from the

capsule reached the medulla, cortical cells were also seen along the trabeculae suggesting their migratory path. Presence of medullary cells in the cortex indicate that they were trapped there during the developmental stages.

Mitotic figures were recorded in all the cortical zones during the prenatal and the postnatal periods. Greep and Deane (1949) and Long (1975) had also described local division and replacement of cells within each cortical zone. However, Chester Jones and Spalding (1954) opined that the new cortical cells arose only from the adherent zona glomerulosa cells of the capsule. Nickerson *et al.* (1969) ruled out any possibility of differentiation of fibroblasts into cortical cells.

The cellular arrangement of various cortical zones recorded here totally agrees with the earlier findings in other domestic animals (Venzke, 1975; Dickson, 1984; Dellmann, 1993).

Features of the zona glomerulosa cells noticed in this study support the findings of Ganguli and Ahsan (1978) and Hakeem *et al.* (1993) in goats. However, Idelman (1970) reported the presence of spherical nuclei without prominent nucleoli in the cells of zona glomerulosa in mammals. In the present study a well defined zona glomerulosa was observed contrary to the findings of Prasad and Sinha (1984) in goats.

Presence of basophilic granules in the cytoplasm of zona glomerulosa cells has been reported by Banks (1981), in ruminants. Similar granules were recorded here also.

A sudanophobic zone of tightly packed cells - the zona intermedia, as observed in the present study had been described in several species of experimental and domestic animals (Cater and Lever, 1954; Ito, 1959; Bloodworth and Powers, 1968; Dickson, 1984; Dellmann, 1993; Ashók *et al.*, 1994a). In the present study this zone was not visible during early postnatal life and became well defined after 120 days of age. Numerous mitotic figures were recorded in this region. However, Mitchell (1948) did not observe any cell division in the corresponding zone in rats.

The irregular, polyhedral or cuboidal cells of the zona fasciculata were found to be arranged in radial cords. Acidophilic cytoplasm of the cells in the outer portion of the zone was foamy. These findings in general agree with those of Idelman (1970), Venzke (1975), Banks (1981) and Dickson (1984) in other domestic animals. The cells with foamy cytoplasm located in the outer portion of the zona fasciculata were referred to as spongiocytes. Dellmann (1993) opined that the foamy appearance of the spongiocytes was caused by the dissolution of lipid droplets during tissue processing.

The zona reticularis contained irregular or polyhedral cells arranged in anastomosing cords without any definite pattern as reported in various domestic animals (Banks, 1981; Dellmann, 1993). Pyknotic nuclei, eosinophilic cytoplasm and the presence of lipofuscin pigments in the zone totally agree with the findings in man (Soffer *et al.*, 1961; Belloni *et al.*, 1987) and other mammals (Idelman, 1970). Idelman (1970) opined that the pigment content increased towards the inner part of the zone, however, no such difference in the distribution of lipofuscin pigments could be recorded in the present study. Further, two types of cells viz. dark basophilic and light basophilic cells as reported by Ganguli and Ahsan (1978) in goats also could not be detected here.

The components of adrenal medulla in goats were found to be similar to those of man (Soffer *et al.*, 1961) and other domestic animals (Banks, 1981). Cytoplasm of the glandular cells contained fine chromaffin positive granules. Soffer *et al.* (1961) confirmed that these chromaffin cells were concerned with the elaboration of catecholamines.

Epinephrine cells were distributed in the peripheral zone and norepinephrine cells in the central zone of the medulla. However, occasional intermingling of these cell types also were noticed. Large cells with indistinct boundaries, deep eosinophilic cytoplasm and eccentric nuclei were identified as

epinephrine secreting cells. Small cells with distinct cell boundaries, clear cytoplasm and centrally located nuclei were confirmed as norepinephrine cells. Similar pattern of distribution of cells and cytological characters have been described in the adrenal medulla of various species of animals including goat (Wood, 1963; Prasad and Yadava, 1973, 1974; Prasad and Sinha, 1981a; Nagra *et al.*, 1989; Ashok *et al.*, 1994a).

The predominance of epinephrine cells over norepinephrine cells observed herein agrees with the finding of Dickson (1984) in different species of domestic animals.

An increase in the number of epinephrine cells from day of birth till 180 days was noticed. This indicates that the medullary cells proliferate throughout life. Tischler *et al.* (1989) reported similar findings in rats. They suggested that the proliferation was mediated by interaction of neurogenic and hormonal signals. Tomlinson and Coupland (1990) also have described hypertrophy and hyperplasia of medullary cells in rats. The chromaffin cells of the adrenal medulla are regarded as modified postganglionic sympathetic neurons in domestic animals (Dellmann, 1993).

Follicles containing colloid material in the adrenal medulla as seen in advanced foetal life were observed postnatally also. Smollich (1965, 1966) had reported similar

follicles in various ruminant species. This has been proved to be the morphological manifestation of the functional stress on medulla.

The adrenal capsule and parenchyma were PAS positive even from the early foetal life. The intensity of the reaction increased with foetal age. The differentiating cells of zona glomerulosa and the medullary cells were weakly positive. Bielanska-Osuchowska (1989a, 1989b) also have reported PAS positive material in the cortical cells of foetal pig. In full term foetuses, capsule, trabeculae and zona reticularis were strongly PAS positive. Zona glomerulosa showed a weak reaction while a moderate reaction was seen in the zona fasciculata and medulla. However, colloid in the medulla was strongly PAS positive. The present findings in the postnatal group agree in general with the findings of Prasad and Yadava (1974), Prasad and Sinha (1981b) and Nagra *et al.* (1989) in various domestic animals, eventhough they did not find any PAS positive material in the zona glomerulosa.

Likewise, the acid mucopolysaccharide content also increased in the cortical cells as the foetal age advanced. The intensity of reaction was stronger in various cortical zones compared to the earlier reports in other domestic animals (Prasad and Sinha, 1981b; Ashok *et al.*, 1994b). This could be probably due to the fact that the earlier experiments

were designed to detect only the strongly acidic mucopolysaccharides at a pH of 0.4, while in the present study even the weakly acidic mucopolysaccharides were stained at a pH of 2.5.

During postnatal life, moderate reaction for acid mucopolysaccharides was recorded in the capsule and outer zona fasciculata. The reaction was more intense in the zona glomerulosa, inner zona fasciculata and zona reticularis. Medullary cells were weakly positive. These are partially in agreement with the findings of Prasad and Sinha (1981b) in other domestic animals. They detected only a weak positive reaction, in the zona glomerulosa. Ashok *et al.* (1994b) did not find any acid mucopolysaccharides in the cortical cells in buffaloes, while the medullary cells were moderately positive.

Eventhough the cortical and medullary cells showed a positive reaction for glycogen in young foetuses, the intensity of the same slightly decreasedin the cortical cells as the pregnancy advanced. Albano *et al.* (1976) reported large accumulation of glycogen in the future cortical cells of rabbit foetuses from day 16 onwards. Idelman (1970) noticed glycogen granules in proximity to the liposomes of the cortical cells in rat embryo. All these suggest that they are concerned with the elaboration and regeneration of lipid resources of the gland.

Glycogen was distributed in the cortex and medulla postnatally with varying intensities. This agrees with the findings of Long and Jones (1967) in man. But Planel and Guilhem (1956) demonstrated glycogen granules in the zona glomerulosa only.

The developing cortical cells showed strong alkaline phosphatase activity upto 84 days of foetal age. There after a moderate activity upto 122 days was observed which then intensified towards term. Wintour *et al.* (1975) proved that between 60 and 90 days of gestation, ACTH was a potent stimulus for aldosterone and cortisol production in foetal sheep. Between 90 and 120 days the effect of ACTH was reported to decline and hence both the glucocorticoid and mineralocorticoid levels were lowered during this period compared to the other periods of gestation. The reduced activity of alkaline phosphatase between 84 and 122 days of foetal life observed in the present study could be due to the reduced activity of the cortical zones during this period, as observed in sheep. A weak activity in the subcapsular zone and total absence in the migratory cells observed here concurs with the findings of Bielanska-Osuchowska (1989a, 1989b) in the foetal pig.

In the postnatal animals, a strong alkaline phosphatase activity was recorded in the cortical cells upto 30 days

followed by a reduced activity at 45 days. Thereafter the activity once again increased in the zona glomerulosa and zona fasciculata and remained without much variation upto 180 days. Upto 45 days all the three cortical zones showed strong activity whereas, after 45 days, the activity was more in the zona glomerulosa. From 60 to 180 days it was strong in the zona glomerulosa and moderate in the zona fasciculata and weak in the zona reticularis. Nicander (1952) observed weak activity in the zona glomerulosa and strong activity in the other two zones in laboratory animals. Similar findings were reported by Arvy (1971) in rabbits, pigs, sheep and goats. However, Bhattacharya and Saigal (1985) and Nanda *et al.* (1993) observed a diminishing intensity of alkaline phosphatase activity in the adrenal cortex of goats, being maximum in the zona glomerulosa as observed in the present study.

The acid phosphatase activity was generally weak in the cortex and strong in the medulla of foetal goats in this study. This observation, in general, agrees with the findings of Bielanska-Osuchowska (1989a) in the foetal pig.

In the postnatal animals the enzyme activity was moderate in the zona reticularis throughout the period of study. Upto 45 days both the zona glomerulosa and zona fasciculata exhibited a strong activity. However, at 45 days it reduced

and remained weak in the zona glomerulosa and moderate in the zona fasciculata till 180 days. Medullary cells revealed a very strong enzyme activity. Nicander (1952) and Penney and Brown (1971) observed a uniform acid phosphatase activity throughout the cortex in cats and rabbits, while a stronger activity in the zona glomerulosa in horses and bovines. The strong activity in the medullary cells agrees with the findings in buffaloes (Ashok *et al.*, 1994b). The present findings in goat with regard to the differences in the intensity of enzyme activity between the epinephrine and norepinephrine cells are in agreement with the observations of Soffer *et al.* (1961) in man and Ashok *et al.* (1994b) in buffaloes.

In this study lipid droplets were detected from 50 days of foetal life itself. Till 74 days, a uniform distribution of lipid was recorded in the cortex. After this it concentrated more in the cluster of cells in the subcapsular region. Jaya and Sulochana (1989) also noticed high concentration of lipid in the future zona glomerulosa cells in human foetus. Albano *et al.* (1976) observed lipid accumulation in the adrenal cortex of foetal rabbits towards the end of gestation only. However, Robinson *et al.* (1979) noted occasional lipid droplets in the cortical primordium between 30 and 60 days of gestation in sheep foetuses.

Between 84 and 122 days of foetal age, lipid content in the cortex other than the zona glomerulosa showed a decrease. Robinson *et al.* (1979) also made a similar observation in foetal sheep. After 122 days lipid accumulation increased in all the cortical zones with a heavy accumulation after 141 days. Yamauchi (1979) also noticed lipid droplets in the equine foetal adrenal, the concentration of which gradually increased towards term. Gutte *et al.* (1986) noticed lipid droplets from 80 days onwards in the adrenal cortex of foetal pigs.

Idelman (1970) stated that the lipid droplets were the precursors of hormones and not necessarily the hormones as such. Deane (1958) and Dickson (1984) were also of the same opinion and they suggested that a decreased lipid content was an indication of increased functional activity of the gland. Nussdorfer *et al.* (1978) and Nussdorfer (1980) proved that chronic stimulation of cortical cells resulted in an increased lipid content after an initial decrease while prolonged depression caused decrease in lipid content after a transient increase. A decrease in lipid content between 84 and 122 days followed by a heavy accumulation towards term was observed in the present study. This might be due to a decreased activity of the gland followed by an increased activity after 122 days under the influence of ACTH as reported by Wintour *et al.* (1975). The stress on the growing foetus stimulated increased

production of ACTH from the anterior pituitary which exerted a prolonged stimulation of the cortical zones (Arthur *et al.*, 1982). Chronic stimulation of the cortical zones by elevated ACTH resulted in the accumulation of lipid droplets. The maximum lipid accumulation in the cortical zones after 141 days of gestation is an indication of extreme stress on the growing foetus due to insufficient nutrient supply and gaseous exchange. An increased enzyme activity coupled with heavy accumulation of lipids after 122 days till term in the cortical cells observed in the present study indicates a higher activity of the gland during the period.

In the postnatal period, there was a heavy accumulation of lipid particularly in the zona fasciculata and zona reticularis in day old and 15 days old animals. Thereafter it decreased at 30 days followed by a heavy accumulation in all the three cortical zones at 45 days. Following this, a gradual depletion in lipid content was noticed from all the cortical zones and remained moderate till 180 days.

Exposure of the neonatal subject to a new surrounding results in extreme stress. This is believed to necessitate more glucocorticoid production from the zona fasciculata under the influence of ACTH. By about 30 to 45 days the young one might be getting acclimatized to the new surroundings thus relieved from the stress. Consequently ACTH release from the

pituitary is believed to diminish. This must be the cause of heavy accumulation of lipid in the cortex by 45 days. Wintour *et al.* (1975), Nussdorfer *et al.* (1978) and Nussdorfer (1980) also derived similar conclusions. Eventhough renin-angiotensin system is the chief controlling pathway of zona glomerulosa, there are evidence to prove that ACTH also play a major role in the regulation and maintenance of zona glomerulosa function and the stimulation of its growth (Nussdorfer, 1980). Hence a heavy lipid accumulation observed in the zona glomerulosa at 45 days postpartum could be due to the reduced ACTH level.

Absence of lipid in the zona intermedia observed in this study is in accordance with the findings of Cater and Lever (1954) in the cat, sheep and rabbit.

Lipid droplets were noticed in the cortical cells surrounding the central vein, in the cells of the cortical islands within the medulla and in the cortical cells found in the capsule. This is in agreement with the earlier findings in goats by Otsuka (1962) and Ganguli and Ahsan (1978).

After 120 days postpartum, the zona glomerulosa contained low levels of lipid while the other two cortical zones contained moderate concentrations as observed in adult buffaloes (Ashok *et al.*, 1994b). Prasad and Sinha (1981b) recorded maximum lipid content in the zona fasciculata and

moderate quantity in the other two cortical zones in various domestic animals including goats. Contrary to this, in Merino ewes maximum lipid concentration was recorded in the zona glomerulosa by Thwaites and Edey (1970).

Till 98 days of foetal life, the medullary cells were exclusively of norepinephrine type. Though a few epinephrine cells were detected at 98 days, a substantial increase was noticed in their number from 129 days to term. However, according to Weiner (1975) the medullary cells during foetal life in rat, rabbit, guinea pig, cattle and man were of the norepinephrine type only. Epinephrine cells began to appear only after birth. Boshier *et al.* (1989) reported division of the medulla into two zones by 100 days of development in sheep foetuses. In both the types of medullary cells only norepinephrine granules were present.

Chromaffin positive cells were recorded in the present study from 50 days of foetal life onwards. Miller (1926) detected catecholamines from the medullary anlage by 15th day of intrauterine life in mouse. Lever (1955) and El-Maghraby and Lever (1980) opined that the time of appearance of positive chromaffin reaction varied between the species of animals.

The iodate positive cells were located more towards the centre of the medulla. This agrees with the report of Wood

(1963) in various species of animals and Ashok *et al.* (1994b) in buffaloes. These iodate positive cells were reported to be norepinephrine type (Hillarp and Hokfelt, 1953; Wood, 1963).

The capsular thickness increased with age during both in the prenatal and postnatal periods. In foetuses, the width of zona glomerulosa, rest of the cortex as well as the entire cortex increased steadily from 78 days to 135 days of gestation. After this, though the entire cortical width as well as the width of the cortex excluding zona glomerulosa showed a significant increase till term, the width of zona glomerulosa slightly decreased. These findings concur with the observations of Boshier *et al.* (1980) in the foetal sheep. Durand *et al.* (1978) noticed a rapid growth of adrenal cortex after 116 days till term in sheep foetuses. However, a midgestational growth of cortex followed by a period of much reduced growth before the rapid growth rate towards the end of gestation described by Boshier and Holloway (1989) in sheep and Lohse and First (1981) in pigs are contrary to the present findings.

Diameter of the medulla also increased steadily from 74 days of gestation till term. Nicolle and Bosc (1990) also recorded increase in the volume of cortex and medulla between 100 and 151 days of gestation in foetal sheep. The present

finding is in total agreement with the observations of Hakeem *et al.* (1993) in crossbred foetal goats.

A sharp increase was recorded in the adrenal weight after 141 days of gestation in goat foetuses. Similar findings were reported in sheep (Comline and Silver, 1961; Thurley, 1972; Boshier and Holloway, 1989) and pig (Lohse and First, 1981) foetuses. This increase could be due to the rapid growth of both the cortex and the medulla during this period as observed in the present study. Boshier *et al.* (1980) also reported a rapid growth phase of the inner cortical zones after 136 days of gestation together with increased catecholamine secretion from the medulla and cortisol secretion from the cortex, in sheep foetuses. In foetal pigs, Lohse and First (1981) noted an increased growth of the zona fasciculata between 105 and 113 days of gestation which was reflected by an elevated cortisol level in the foetal plasma during the period. In foetal sheep, Nicolle and Bosc (1990) have reported hypertrophy and hyperplasia of cells in the cortex especially in the zona fasciculata after 132 days. In the medulla such changes were observed after 144 days. Foetal pituitary was reported to exert a direct influence on the cortical growth, particularly on the zona fasciculata.

While studying the proportionate growth of the zona glomerulosa and the rest of the cortex in foetus, it was found

that the zona glomerulosa/rest of the cortex ratio showed a significant decrease towards term after a marginal increase between 78 and 135 days. This decrease was found to be due to the faster growth rate of the rest of the cortex and a reduced growth rate of zona glomerulosa. It could be inferred that the portion of the cortex excluding the zona glomerulosa particularly the zona fasciculata was more active during the terminal stages of gestation.

When the growth rates of cortex and medulla were compared, the cortex/medulla ratio showed an increasing trend from 74 days to term. This indicates that the cortex has a faster growth rate. Present findings agree with the observations of Boshier *et al.* (1989) in sheep that the percentage contribution of the medulla to the entire adrenal declined during the end of gestation and the perinatal period due to a rapid cortical growth. This could be under the influence of ACTH as suggested by Wintour *et al.* (1975). However, in equine foetuses Yamauchi (1979) recorded a declining trend in the ratio as the pregnancy advanced.

After birth, the width of the various cortical zones other than the zona reticularis increased from the day of birth to 180 days with a slight decrease at 45 days. The zona reticularis, after an initial increase, decreased in width towards 180 days of age. Excepting the width of zona

reticularis, all other parameters had a positive correlation with age. These variations in the dimensions of the cortical zones must be the results of environmental stress on the adrenal cortex. A higher activity of the gland till 30 days followed by a much reduced activity at 45 days was evident from the morphometry, micrometry and the histochemical studies.

Diameter of the medulla also showed an increasing trend during the postnatal period. No decrease was observed at 45 days as seen in cortex. Hakeem *et al.*(1993) also noted an increase in the width of zona fasciculata and medulla. Contrary to the present findings they reported a decrease in the width of zona glomerulosa from first to ninth month postpartum in crossbred goats.

The ratio of zona glomerulosa/zona fasciculata increased upto 60 days, decreased between 90 and 120 days and then increased marginally till 180 days. This was due to the fact that the growth rate of zona glomerulosa was comparatively faster upto 60 days and thereafter slowed down. After 60 days the growth rate of zona fasciculata exceeded that of zona glomerulosa upto 120 days after which both the zones had almost the same growth rate. At 30 and 45 days a lower width of zona fasciculata also would have contributed to a higher ratio during this period.

The cortex/medulla ratio showed a decreasing trend upto 45 days followed by a marginal increase upto 180 days. This shows that the medulla grew at a faster rate than the cortex upto 45 days. Thereafter the growth rates of both the zones were almost on par with each other till 180 days. Similar data are not available for comparison.

In the present study, it was found that the increased adrenal weight during prenatal and postnatal periods was the result of both the cortical and medullary growth. In foetal period, the cortical growth occurred mainly in the inner part of the cortex excepting the zona glomerulosa. These findings are in conformity with the reports of Boshier *et al.* (1980) in foetal sheep. After birth, the increased adrenal weight was mainly attributed to the growth of zona fasciculata among the different cortical zones.

The various micrometrical and physical parameters were significantly higher in the postnatal groups. Moreover, histologically no regressive change could be detected either in the cortex or in the medulla during the period of study. From these it can be concluded that `foetal cortex' does not exist in goats as seen in the primates.

The percentage contribution of the zona glomerulosa to the entire cortex was more in foetal period compared to the postnatal animals. In this study, the zona glomerulosa

contributed 16.71 per cent, zona fasciculata 74.16 per cent and *zona reticularis* 9.13 per cent to the total cortical width, in the postnatal group. In dogs Hullinger (1978) reported 27 per cent zona glomerulosa, 50 per cent zona fasciculata and 23 per cent zona reticularis, while in camels it was 18 per cent, 53 per cent and 29 per cent (Abdalla and Ali, 1988-89). Hakeem *et al.* (1993) recorded slightly higher value for zona reticularis and lower values for zona glomerulosa and zona fasciculata in goats. These values indicate that proportion of various zones differ among species.

Summary

SUMMARY

In crossbred goats, the adrenal glands were located cranial to the kidneys in the retroperitoneum. The right gland was roughly triangular while the left one was slightly elongated.

During the prenatal period, weight of the adrenals increased and a spurt was noticed after 141 days of gestation. The glandular weight was positively correlated with age and body weight. But the adrenal to body weight percentage showed a decreasing trend with the age. The spurt in the adrenal weight towards term was concluded to be due to the effect of enhanced foetal ACTH production resulting from the increased stress on the growing foetus.

Postnatally also the adrenal weight showed an increasing trend with a decrease at 45 days. The increased weight of the glands during early neonatal periods followed by a reduced weight at 45 days could be due to the acclimatization of the young one to the new surroundings subsequent to the effect of heavy stress on the new born which are exposed to the external environment at birth. The decreasing trend in the proportion of adrenal weight to body weight percentage during the study period indicated that the adrenal growth rate was not in proportion to the body growth rate.

The left adrenal was slightly heavier and longer than the right while the right one was broader and thicker. The length, breadth and thickness of the glands showed an increasing trend throughout the period of study with a slight decrease at 45 days postpartum. All these parameters were positively correlated with age.

The adrenal anlage was first detected in 33 days old embryos as whorls of cells. Primordial cells began to organize into cords and dense aggregations by about 36 days of development.

A thin capsule composed of collagen fibres was detected around the primordium by 42 days of gestation, division of which into outer more fibrous and inner more cellular layers was visible by 70 days. Reticular fibres were recorded in the capsule by 58 days and elastic fibres by 74 days.

Histological differentiation of the gland started by 42 days with organization of the cells into small groups and cords separated by irregular spaces. First sign of central vein formation was also noted at this stage. The glands were clearly demarcated from the surrounding structures by about 50 days.

The migration and penetration of future medullary cells into the cortical primordium started by 50 days and was

completed by 70 days. The norepinephrine cells and the chromaffin reaction were detected from 50 days of foetal life. However, epinephrine cells showed their presence by 98 days only and their number increased steadily towards term. Increased number of epinephrine cells and a rapid growth of zona fasciculata were noticed towards the end of gestation.

Organization of zona glomerulosa started by 58 days and completed by 95 days. Zona fasciculata and zona reticularis were completely differentiated by 129 days of foetal life. Separation of cortex and medulla started by 70 days and both were clearly demarcated by 95 days. However, groups of cortical cells were seen as islands in the medulla and also around the central vein during both prenatal and postnatal periods.

Follicles containing colloid material were frequently encountered in the medulla both in the foetuses of advanced stages of gestation and in the postnatal animals. They have been reported to be histological manifestations of extreme functional stress on the adrenal medulla.

In the postnatal animals, the capsule was highly vascular and composed of collagen, reticular and elastic fibres with a few smooth muscle cells. An outer more fibrous and an inner more cellular layers were distinguishable. The capsule

contained groups of undifferentiated cells, islands of cortical cells, fibroblasts, neurons and melanocytes.

The parenchyma was divisible into an outer cortex and an inner medulla. The cortex consisted of a zona glomerulosa, zona fasciculata and zona reticularis. Cells in each zone had definite pattern of arrangement and possessed distinctive characteristics.

The cells of the zona glomerulosa were polyhedral with vesicular nuclei. Cytoplasm was vacuolated and contained basophilic granules. These cells were arranged in irregular clusters and cords. The irregular, polyhedral or cuboidal cells of the zona fasciculata were arranged in radial cords. Acidophilic cytoplasm of the cells in the outer portion of the zone was foamy and were referred to as spongiocytes. The irregular or polyhedral cells of the zona reticularis were arranged in anastomosing cords without any definite pattern. Some of these cells had pyknotic nuclei. The cytoplasm was eosinophilic and contained lipofuscin pigments. The acidophilia of the cytoplasm gradually increased from the zona glomerulosa to the zona reticularis. Mast cells were recorded occasionally in all the three cortical zones. The trabeculae from the capsule at times traversed the entire cortex and reached the medulla. Mitotic figures were recorded in all the cortical zones during the postnatal period also.

The adrenal medulla contained glandular cells possessing chromaffin granules, neurons, trabeculae and sinusoids. The cells of the outer zone - the, ~~nor~~epinephrine cells, were larger with indistinct boundaries, deep eosinophilic granular cytoplasm and eccentric nuclei and were arranged mostly in cords. The smaller norepinephrine cells of the inner zone showed distinct cellular boundaries, clear cytoplasm and centrally placed nuclei. The former type revealed brownish purple cytoplasm while the latter was stained yellow with Wood's technique. Epinephrine cells increased in number from the day of birth upto 180 days indicative of medullary cell proliferation throughout the life.

The capsule, trabeculae and parenchyma were PAS positive with varying intensities. Acid mucopolysaccharide was also seen throughout the gland. Intensity of both increased with foetal age. Glycogen was detected in the cortical and medullary cells, the intensity of which slightly decreased towards term. During the postnatal period glycogen content was more in the zona glomerulosa and zona reticularis than in the zona fasciculata and medulla.

During foetal life alkaline phosphatase activity was noticed in the cortical cells, the intensity of which varied with foetal age indicating the functional status of the gland. In the postnatal period intensity of the enzyme activity

diminished from the zona glomerulosa to the zona reticularis. High activity was seen upto 30 days followed by a much reduced activity at 45 days especially in the zona fasciculata. A moderate alkaline phosphatase activity was detected in the medulla.

After 122 days of gestation cortical cells revealed a more intense acid phosphatase activity compared to the early stages. During postnatal period initially a high activity was noticed upto 30 days the intensity of which diminished by about 45 days and continued upto 180 days. Intensity of the reaction varied among the different cortical zones. Medullary cells revealed higher acid phosphatase activity compared to the cortical cells in both the groups. Peripheral zone of epinephrine cells had a higher activity than the central zone of norepinephrine cells.

Lipid was first detected at 50 days of foetal life. Between third and fourth month of gestation there was a reduction in the lipid content, after which a steady rise in its concentration was noticed. This reached a peak during the last two weeks of pregnancy. This indicated the functional status of the gland.

In the postnatal period also lipid levels fluctuated in the cortical zones. Initially a high level was noticed upto 15 days and then a slight decrease at 30 days. Maximum amount

was detected at 45 days throughout the cortex. After this a slight depletion was noticed and thereafter the concentration did not vary much.

Capsular thickness, width of the entire cortex and that of the various cortical zones and diameter of the medulla were positively correlated with age in the prenatal group. However, in the postnatal group, the zona reticularis had a negative correlation with age, eventhough other parameters were positively correlated.

Cortical width increased throughout the foetal period, with a spurt during the last two weeks of pregnancy. Medullary diameter showed a steady increase throughout the foetal period. The marked increase noticed in the adrenal weight towards the end of gestation can be attributed to the high growth rate of cortex and medulla during this period. Cortical growth rate was found to be much higher than that of the medulla.

After birth also an increasing trend in the width of the cortical zones and diameter of the medulla was noticed. However, at 45 days, width of the zona fasciculata decreased due to a diminished activity. Growth rate of zona glomerulosa was higher than that of zona fasciculata till 60 days, after which the latter grew faster. The medulla grew faster than

the cortex upto 45 days and thereafter no significant difference in growth rates could be detected.

Results of morphometry, micrometry and histochemical studies revealed that the adrenal cortical activity especially of the zona fasciculata remarkably increased on nearing parturition. Foetal stress at the end of gestation caused by insufficiency of the placenta might be the reason for the higher activity of the adrenal cortex during this period. After birth, an increased cortical activity was observed till 30 days followed by a reduced activity at 45 days. At parturition, exposure of the young one to the new environment induces stress and this could be responsible for a higher activity of the cortex during the early neonatal periods. Once the young one gets acclimatized to the external environment, effects of stress are removed, thus cortical activity again gets reduced by about 45 days.

The increase in adrenal weight during prenatal and postnatal periods was contributed by both cortical and medullary growth. In the foetal period, the cortical growth occurred mainly in the inner part of the cortex excepting the zona glomerulosa.

The `foetal cortex' reported in primates was not detected in goats.

Percentage contribution of zona glomerulosa to the entire cortex was more during the foetal period compared to postnatal animals. In goats, on an average the zona glomerulosa contributed 16.71 per cent, zona fasciculata - 74.16 per cent and zona reticularis - 9.13 per cent to the total cortical width.

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DEVELOPMENT OF THE ADRENAL GLAND IN THE CROSSBRED GOAT

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ABSTRACT OF A THESIS

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ABSTRACT

Studies on the prenatal and postnatal development of the adrenal gland in crossbred goats were conducted using 55 embryos/foetuses of varying gestational ages and 45 goats from day old to 180 days postpartum. The project was taken up to trace the normal growth and developmental pattern of the glands and their relationship with age, body weight and the physiological status.

After recording gross relations and measurements, the material was fixed using various fixatives for studying the cellular details, arrangement of cells, connective tissue framework, micrometry and histochemistry.

The glands attained their characteristic shapes even during early development and were located cranial to the kidneys in the retroperitoneum. During the prenatal period weight of the adrenals increased steadily upto 141 days followed by a spurt thereafter due to the hypertrophy and hyperplasia of the cortex. After birth also an increasing trend was noticed in adrenal weight from birth to 180 days with a slight decrease at 45 days. The glandular weight was positively correlated with age and body weight. The proportion of the adrenal to body weight percentage showed a decreasing trend in both the groups studied. The left gland was slightly heavier and longer than the right while the right one was broader and thicker. The length, breadth and

thickness were positively correlated with the age of foetuses and kids.

The cortical primoridum was first detected at 33 days of embryonic development. By 36 days, these cells began to organize into cords and dense aggregations. Though a thin collagenous capsule began to develop by 42 days, it became conspicuous with outer more fibrous and inner more cellular layers by 70 days. Reticular fibres appeared by 58 days and elastic fibres by 74 days of foetal life.

By 42 days, histological differentiation started with organization of cells into small groups and clusters separated by irregular spaces. The central vein also started development at this stage. Differentiation of zona glomerulosa was noticed by 58 days and was completed by 95 days. Zona fasciculata and zona reticularis became apparent by 129 days. A distinct corticomedullary junction appeared at 95 days eventhough interdigitations of cortex and medulla were seen at the junction throughout the study period. Towards the centre of the gland intermingling of cortical and medullary cells were seen upto 104 days. Patches of cortical cells were seen in the medullary region and also around the central vein throughout the period of study. Neural crest cells invaded the cortical primordium by 50 days and this process was completed by 70 days. Chromaffin reaction appeared in the medullary cells by 50 days. Eventhough norepinephrine cells were detected in the gland at this stage, epinephrine cells made their presence only by 98 days. Follicles containing

colloid material were encountered in the medulla during both the advanced foetal and the postnatal periods.

In goats, the glands were covered by highly vascular connective tissue capsule composed of collagen, reticular and elastic fibres with a few smooth muscle cells. An outer more fibrous and an inner more cellular layers were recognizable. The capsule contained undifferentiated cells, differentiated cortical type cells, fibroblasts, neurons and melanocytes.

The parenchyma was divisible into a cortex and a medulla. Cortex was further subdivided into zona glomerulosa, zona fasciculata and zona reticularis. Each zone had distinct pattern of cellular arrangement and cytological characteristics. Mast cells were occasionally detected in all the cortical zones. Mitotic figures were also recorded throughout the cortex. A zona intermedia was observed between the zona glomerulosa and the zona fasciculata.

Capsule, trabeculae and the parenchyma were all PAS positive. Acid mucopolysaccharides and glycogen were also detected in the cortex. They were seen at varying intensities in the cortex and medulla of the prenatal and the postnatal subjects.

Intensity of phosphatase enzymes was lower in the cortical cells between third and fourth month of gestation after which the same increased till term. After birth a higher activity upto 30 days and a reduced activity at 45 days were recorded especially in the zona fasciculata. Medullary

cells revealed moderate alkaline phosphatase and intense acid phosphatase activities during the study period.

Lipid was first detected by 50 days, and upto 74 days a uniform distribution was seen throughout the cortex. Afterwards, it concentrated more in the clusters of cells in the subcapsular region. Between 84 and 122 days, a low lipid content was noticed in the cortical cells, the concentration of which gradually increased towards term. After 141 days a heavy accumulation was observed in the cortex. During the postnatal period, the inner two cortical zones showed heavy lipid accumulation upto 15 days and a slight depletion at 30 days. This was followed by a very heavy accumulation in all the three cortical zones at 45 days. Following this, a gradual depletion was noticed from all the cortical zones.

In the prenatal group, capsular thickness, width of the entire cortex as well as the various cortical zones and diameter of the medulla showed positive correlation with foetal age. In the postnatal group, excepting the width of *zona reticularis*, all other parameters were positively correlated with age. After 135 days of gestation, width of the cortex, especially of the inner two cortical zones increased significantly till term.

During postnatal period, a decrease was noticed in the width of outer two cortical zones by 45 days, however the diameter of medulla increased steadily upto 180 days. Growth rate of zona glomerulosa was higher than that of zona

fasciculata till 60 days, after which the latter grew faster. The medulla grew faster than the cortex upto 45 days and thereafter no significant difference in growth rates could be detected.

Percentage contribution of zona glomerulosa to the entire cortex was more during the foetal period compared to the postnatal animals. On an average, during the postnatal period the zona glomerulosa contributed 16.71 per cent, zona fasciculata 74.16 per cent and zona reticularis 9.13 per cent to the total cortical width.

It was concluded that the stress induced on the growing foetus towards the end of gestation due to the insufficiency of placenta resulted in a higher activity of the cortex, particularly of the zona fasciculata under the influence of foetal ACTH. At parturition, exposure of the new born to the external environment induced severe stress which was responsible for the higher activity of the gland during early neonatal period. When the young one got acclimatized to the new surroundings, the stress was relieved which in turn resulted in the reduced activity of the gland by 45 days postpartum.