CHAPTER 6

HISTOLOGICAL STUDIES OF GONADS AND EXTRA-GONADAL TISSUES (*VIZ.* LIVER, INTESTINE AND KIDNEY) OF JUNGLE BABBLERS.

INTRODUCTION~

Reproduction is a biological phenomenon which exhibits a regular recurrence of pattern of activities in a cyclic manner (Lofts and Murton, 1973). Therefore, the reproductive functions exhibit meticulous regulation of initiation and development of gonadal functions which is now known to be a balanced interaction of environmental and physiological states (Phillips *et al.,* 1985).

While studying variations in biochemical and hormonal parameters, though breeding testes were found from April to November non-breeding testes were also found frequently. However from December to February no breeding testes were obtained *i.e.* all the males were in non-breeding state during this period. Male Jungle Babblers show peak testes cycle from April to November.

In females a characteristic feature was observed wherein many females had large ovaries with higher ovarian weights as well as oviducal weights but few of these had ovaries with same size as found in breeding female, but with significantly lower oviducal weights (Chapter 2). These females were categorized as "helpers". Female Jungle Babblers showed a longer breeding season from March-December with occasional regressed ovaries in between these months. As described in earlier chapters there does not exist prominent differences in the biochemical profile of extra-gonadal tissues of male and female Jungle Babbler with helper females. To support these histological studies were also carried out of gonadal and extra-gonadal tissues in different categories of Jungle Babblers described earlier.

Birds are the best known relatively large and adaptively diversified group of vertebrates and detailed information is available regarding their reproductive patterns. The breeding season of Jungle Babblers, seem to differ slightly in different parts of India (Ali, 1993; Whistler, 1949; Andrews, 1968).

Jungle Babbler is a very common bird around which has kept up its originality of reproductive rhythms without undergoing a modifying influence of urbanization. In this chapter, the histological changes in the annual cycles of testes and ovaries along with the histology of extra- gonadal tissues *viz.* liver, intestine and kidney in Jungle Babblers are presented.

MATERIAL AND METHODS~

The birds were procured during the year September 1997 to July 2000. They were kept in an aviary with food and water *ad libitum*. To minimize the stress, birds were sacrificed as soon as possible. Testes and ovaries along with the extra-gonadal tissues like liver, intestine and kidney were taken out and were blotted free of tissue fluid and fixed in Bouin's fluid. Blocks were prepared by following the routine procedure by dehydrating in alcohol grades, clearing the tissue in toluene and embedding in paraffin wax. Blocks were sectioned at 5 ì and stained with Heamatoxylin – Eosin stain for the microscopic studies.

The following measurements were also made on occulo-micrometer for male birds:

1. Diameter of atleast 20 seminiferous tubules (in t.s.) of both breeding and non-breeding males.
2. Thickness of the germ layers of atleast 20 seminiferous tubules (in T.S) of both breeding and non-breeding males.
3. Diameter of interstitium of atleast 20 seminiferous tubules (in t.s.) of both breeding and non-breeding males.

For female birds:

1. The increase and/or decrease in the percentile of different types of follicular cells (*viz.* small, medium, large and atretic follicles).
2. Diameter of the largest follicle.

RESULTS and DISCUSSION~

The average thickness of diameter of the seminiferous tubule, average thickness of the germ layer and the average diameter of the interstitium with body weight, testes weight and GSI for breeding and non-breeding males is given in Table 1 whereas body weight, weight of the ovary, weight of oviduct with GSI and different follicle sizes are given in Table 2. The histological features of testes are given in Plate I and of ovary in Plate II and that of liver, intestine and kidney in Plates III and IV.

TESTES

Spermatogenesis is reported to be fairly conserved process throughout the vertebrate series (Pudney, 1995). Spermatogonia develop into spermatocytes that undergo meiosis to produce spermatids which enter spermiogenesis where they undergo a morphological transformation into spermatozoa. In the amniotes (reptiles, birds and mammals), spermatogenesis occurs in the seminiferous tubules, that possess a permanent population of sertoli cells and spermatogonia which act as a germ cell reservoir for succeeding bouts of spermatogenic activity (Pudney, 1995).

The right testis of Jungle Babbler was always found to be larger in size and higher in weight than the left one. These differences were more pronounced during the breeding season. A regular well defined spermatogenic cycle as reported by Andrews (1968) was also found in the present study. The spermatogenic cycle of the Jungle Babbler is divided into the following stages:

Stage I. Resting spermatogonia only

Stage II. Dividing spermatogonia with a few spermatocytes.

Stage III. Many spermatocytes.

Stage IV. Spermatids and spermatozoa.

Stage V. Many spermatozoa.

Stage VI. Full spermatogenic activity with many spermatozoa still attached to the tubular wall.

Stage VII. Regressing testis.

Most of the breeding testes with spermatids and spermatozoa (Plate I, 4) were found from May to November indicating that Jungle Babbler are in readiness to breed for long period from May to November. However, non-breeding testes with resting spermatozoa were also found all throughout the year (Plate I, 1). In breeding testes the diameter of the seminiferous tubule was 55.25 $\pm$ 0.11ìm. When spermatids and spermatozoa were present (Plate I, 4) the seminiferous tubule diameter was larger than stage in which spermatozoa were still attached to germ layer (Plate I, 6). Maximum diameter is observed in seminiferous tubule with primary and secondary spermatocytes.

The average thickness of germinal cell layer in breeding and non-breeding testes were 16.25 $\pm$ 0.09 μm and 8.12 $\pm$ 0.088 μm respectively. The average interstitium diameter in breeding and non-breeding males was 4.72 ± 0.03 μm and 10.72 ± 0.031 μm respectively (Table 1). In the regressing testes the rows of spermatogonia and occasionally a few spermatocytes were seen.

The avian testicular interstitium is reported to be similar to that of the human and cat in possessing a multilayered myofibroblast component and that of the rodent in possessing a small number of Leydig cells as well as in the location of the lymphatic vessels. The bird combines characteristic of the interstitium found variably in mammals (Aire, 1997) with centrally located blood vessels and Leydig cells as observed in Jungle Babblers too (Plate I, 3). The basal lamina resting on a closely associated homogenous microfibrillar layer can be seen in Plate I (2, 3, 4, and 6). The basal lamina in resting testes was not distinct. According to Aire (1997) the basal lamina in non-breeding testes is relatively electron dense, contained globules and sent numerous fingers like processes into the seminiferous epithelium, particularly into the sertoli cells. In Jungle Babbler the Leydig cells were distinct in all stages except the stage when spermatozoa are present still attached to the germ layer (Plate I, 6).

The interstitial tissue of breeding testes is known to be tightly packed with the Leydig cells which contain relatively large amounts of lipid droplets related to androgen synthesis; while that in the non - breeding testes, the interstitial tissue contains only occasional Leydig cells with an enlarged intercellular space. These Leydig cells contain small amounts of ER mainly RER and low levels of androgen (Rosenstrauch, 1998).

OVARY

The various follicular stages observed in the ovary of Jungle Babbler in present study were categorized as small follicles, medium follicle, large follicles and atretic follicles. These stages were noted for all the three females' *viz.* breeding and non-breeding females along with the helper females. The percentage of these follicular stages in the above mentioned females are given in table 2. The percentile of small follicles present in breeding, non-breeding and helper females were 4, 17 and 8 respectively, whereas the percentage of medium sized follicles were 12, 5 and 10 respectively. The large sized follicles were seen maximum in breeding females at 10% followed by helper females at 4% and least were observed in the non-breeding females at 2%. The atretic follicles were observed maximum in helper females at 8% followed by non-breeding and breeding females at 6% and 5% respectively. The mean diameter of the largest follicle present in the breeding females was 8.90mm while that in helper females was 5.60mm and least follicular diameter was observed in non-breeding females at 1.98mm.

The structure of the avian ovary has been described by several workers (Hodges, 1975; Nalbandov, 1970; Sturkie, 1986). The avian ovary consists essentially of an outer cortex containing ova which surrounds a highly vascular medulla composed primarily of connective tissue. The surface of the cortex is covered by the cuboidal germinal epithelium. There is vast number of ova developing in the ovary but only few reach maturity and only a comparative few are ovulated. The surface of the ovary is covered by the germinal epithelium consisting of a single layer of cells. All the ova within the ovary are primary oocytes until before ovulation. Within the cortex there are numerous minute developing ova. In Jungle Babbler also similar stages were found. The active ovary consisting of number of large follicles which are passing through the final stages of yolk accumulation is shown in Plate II a, 2. The follicle consists of number of layers, the theca externa, the theca interna, the basement membrane, the membrana granulosa and the perivitelline membrane as shown in Plate II a, 1& 2.

The theca externa is known to comprise the greater part of the thickness of the follicular wall and contains muscle fibers. In birds the theca interna is a much thinner layer (Plate II a, 2) which consists of collagen fibers. Basement membrane is a layer of cells separating the theca interna from the membrana granulosa. The follicular epithelium (membrana granulosa) is a layer immediately adjacent to the ovum (Plate II a, 2). Zona radiata is the most peripheral region of the oocyte. Atretic follicles are known to occur normally and regularly in active ovaries. When follicles reach the maximum size, the granulosa cells begin to proliferate forming numerous irregular layers around the ovum. The ovum size decreases and eventually granulosa cells fill the entire follicle (Plate II b, 4). This undergoes hypertrophy and becomes a connective tissue scar. Immediately after ovulation the follicle shrinks and due to this the walls become thickened and granulosa increases to several cells in thickness. The follicle regresses rapidly and is eventually reabsorbed into the mass of the ovary.

A histochemical study has been made of seasonal fluctuations in the follicular atresia and interstitial gland tissue with the ovarian cycles of the house sparrow (Guraya and Chalana, 1976) in crow and myna (Chalana and Guraya, 1979) in grey quail (Saxena and Saxena, 1980) in house swift (Naik and Naik, 1965). The atresia of the primordial oocyte forms the predominant feature of the quiescent-winter ovary. The building up of interstitial gland tissue of the thecal origin, which precedes the breeding activity, is closely related to the atresia of previtellogenic follicles of variable sizes (Chalana and Guraya, 1979).

LIVER

The traditional unit of liver structure, the lobule, which is seen in mammals is not very evident in birds due to the lack of distant inter lobular septa. Thus, the livers shows more or less homogenous mass of liver cords, composed of liver parenchyma with intra lobular veins and inter lobular vessels arranged irregularly through out (Hodges, 1975). As seen in Plate III, 1 in Jungle Babblers also no true lobular framework of basic vertebrate liver structure is seen in Jungle Babbler and it is composed of a mass of cords and sinusoids. The surface of the liver is covered by a peritoneal membrane. The hepatic cells appear to be roughly triangular in shape (in t.s.) with several usually 4 to 6 grouped bile canaliculi. With in the hepatocytes is a large spherical nucleus.

In mammals and higher birds, the hepatic plates are one cell thick but in primitive birds the hepatic plates are one or two cell thick (Hickey and Elias, 1954). The Jungle Babbler is probably among the primitive birds in which the hepatic Plates are two cells thick.

KIDNEY

In birds each kidney is elongated somewhat irregular in shape and dark brown in color present in the dorsal abdominal area. Ventrally the kidney consists of three regions usually termed as lobes: cranial, middle and caudal. The basic unit of the kidney is lobule which is irregular, polyhedral areas and lobule consists of cortical and medullary tissue (Plate III; 2, 3, & 4). The nephrons are arranged between the inter lobular and intra lobular veins with the glomerulae lying roughly midway between the two. The glomerulus close to the medulla is larger than those in peripheral region. The cortical tissue lying between the glomerulae and the inter lobular veins consists of proximal convoluted tubule whilst the area between the intra lobular veins is composed of distal convoluted tubules. Though a typical lobular pattern with cortical and medullary region of avian kidney exists in Jungle Babblers, the lobules are not as distinct as observed in other species of birds like Bank Myna and Brahminy Myna (Sapna 2002).

There does not occur seasonal variability in kidney morphology of Jungle Babblers. Casotti (2001), have also reported that no variation in the volume of the structure within the kidney occur between the seasons. The only variable to show difference was volume of distal convoluted tubule and cortical connective tissue in House sparrow. Thus, kidney morphology is relatively unaffected by the changes in the season or the reproductive status.

INTESTINE

The avian intestine follows the basic vertebrate pattern of outer most layer of connective tissue called peritoneum followed by muscularis externa with longitudinal and circular muscles; submucosa and mucosa. However in birds the muscularis externa is not well developed. As also seen in Jungle Babbler the circular muscle is poorly developed compared to longitudinal muscle layer. It is generally considered that Brunner's gland do not occur in birds, however, Hodges (1975), has recognized such glands in the several specimen of fowl. In Jungle Babbler a prominent narrow zone of glands can be seen in (Plate IV; 1 & 4). In Jungle Babbler, in the duodenal part the villi are blunt and with prominent large lacteals- the lymphatic capillary. In mammalian small intestine the lacteals begins in the villus as a blind capillary and drains into larger lymphatic vessels in the submucosa (Ross and Reith, 1985). After the meals the lacteals are dilated and readily evident. Jungle Babblers spends most of their time in feeding hence, probably the lacteals are always dilated. This needs further investigation with reference to the feeding and the histological changes in the different parts of alimentary canal in a bird like Jungle Babbler. Lamina propria extending in the core of villi probably becomes thin and gets pushed on one side.

Table: 1 The histological changes in the average diameter of the seminiferous tubule, average thickness of the germ layer and the average diameter of the interstitium.

	Body weight (in gms)	**Testes weight (in mgs)**	**GSI**	**Avg. diameter of Seminiferous tubule (in μ)**	**Avg. thickness of germinal layer (in μ)**	**Avg. diameter of interstitium (in μ)**
Breeding males	61.4 ± 2.27	205.0 ± 14	0.326 ± 0.032	55.25 ± 0.11	16.25 ± 0.09	4.72 ± 0.03
Non-breeding males	56.7 ± 2.24	13 ± 1	0.025 ± 0.004	24.37 ± 0.1	8.12 ± 0.088	10.72 ± 0.036

Explanations to Figures:

Plate I

Spermatogenic cycle in male Jungle Babbler

(Turdoides striatus)

Stage I. Resting spermatogonia (800 X).

Stage II. Dividing spermatogonia with a few spermatocytes (800 X).

Stage III. Many spermatocytes (800 X).

Stage IV. Spermatids and spermatozoa (800 X).

Stage V. Many spermatozoa (800 X).

Stage VI. Full spermatogenic activity with many spermatozoa still attached to the tubular wall (500 X).

Stage VII. Regressing testis (800 X).

S: spermatogonia, **St:** spermatocytes, **Lc:** Leydig cells,

BV: Blood vessel, **Sp:** Spermatids, **Sz:** spermatozoa, **L:** Lumen.

ST: Seminiferous tubule. **I:** Interstitium

Plate I

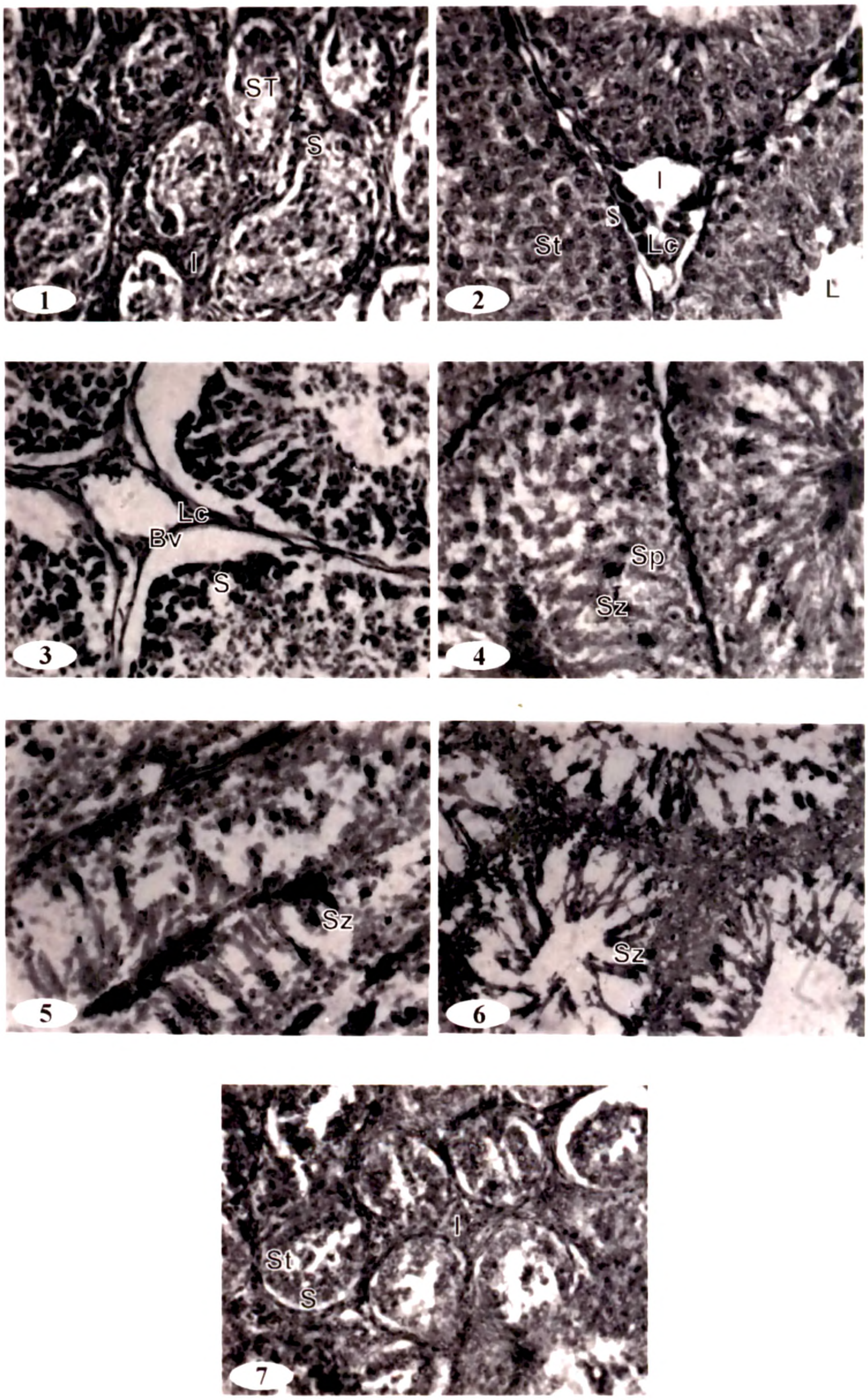


Table: 2 Variations in GSI and size of the follicles in female Jungle Babblers.

	Body weight (in gms)	Ovary weight (in mgs)	Oviducal weight (in mgs)	GSI	Small follicles	Medium follicles	Large follicles	Atretic follicles	Mean diameter of largest follicle
Breeding female	58.66 ± 4.5	72.66 ± 9.39	181.66 ± 30.17	0.129 ± 0.024	4%	12%	10%	5%	8.90
Non-breeding female	54.0 ± 2.47	9.0 ± 1.27	8.71 ± 2.87	0.028 ± 0.011	17%	5%	2%	6%	1.98
Helper female	58.14 ± 1.86	65.71 ± 6.03	47.57 ± 8.30	0.113 ± 0.010	8%	10%	4%	8%	5.60

Explanations to Figures:

Plate II a

Ovary of breeding Jungle Babbler *(Turdoides striatus)*

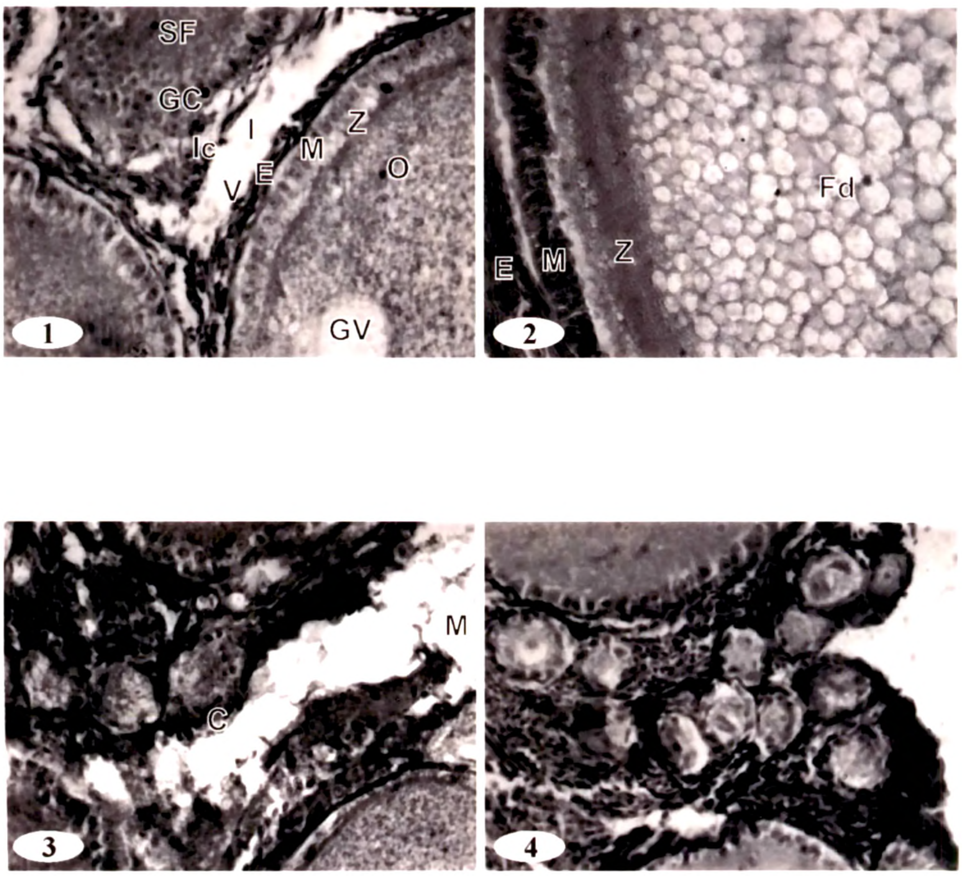
Fig. 1. Ovarian follicles of large and medium size (800 X).

Fig. 2. Enlarged (large) follicle, within ova fat droplets (Fd) (800 X).

Fig. 3 & 4. Cortex with many small sized follicles (800 X).

SF: Secondary follicle, **O:** Oocyte, **GC:** Granulosa cells, **GV:** Germinal vesicle, **Ic:** Interstitial cells, **Z:** Zona radiata, **M:** Membrana granulosa, **V:** Theca externa, **M:** Medulla, **C:** Cortex

Plate IIa



Explanations to Figures:

Plate II b

Ovary of helper and non-breeding Jungle Babbler

(Turdoides striatus)

Fig. 5. Many small and medium sized follicles (500 X).

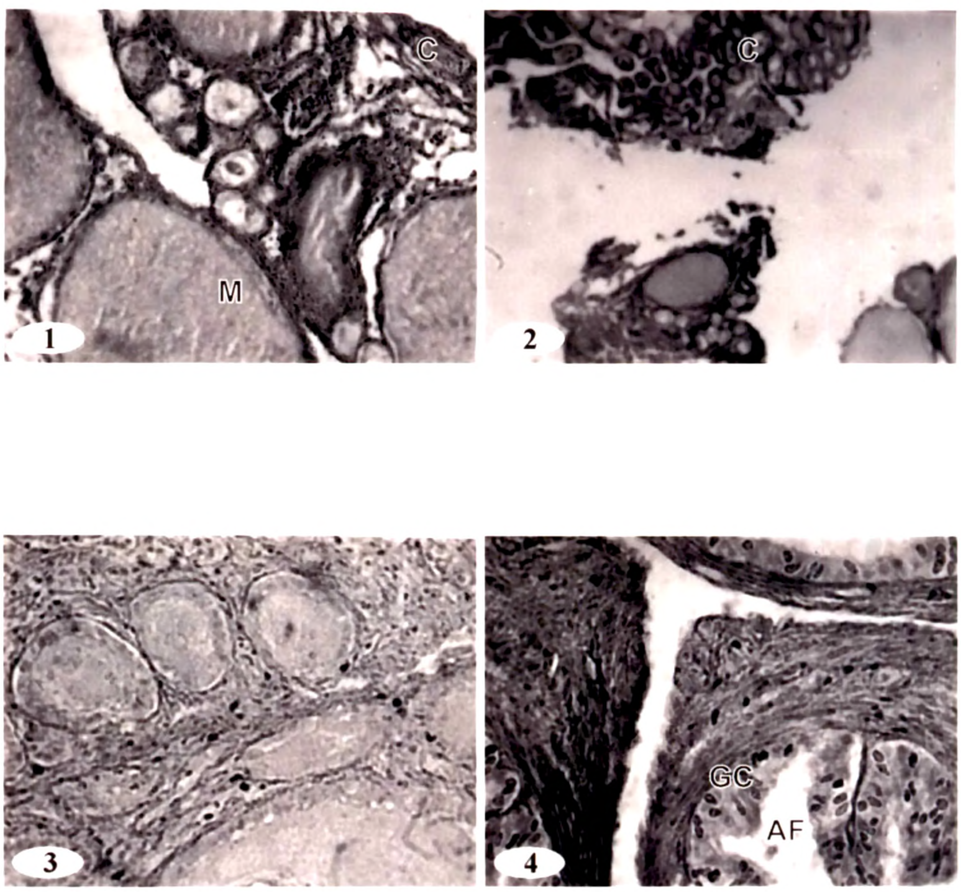
Fig. 6. Cortical region with many small follicles (200 X).

Fig. 7. Non-breeding ovary with large number of small follicles (800X).

Fig. 8. Atretic follicle (AF) with cellular mass invading follicular cavity (C) (800 X).

AF: Atretic follicle, **GC:** Granulosa cells

Plate IIb



Explanations to Figures:

Plate III

Liver of Jungle Babbler *(Turdoides striatus)*

Fig. 1. H: Hepatocytes, **Cc:** Central vein, **S:** Sinusoids (800 X).

Kidney of Jungle Babbler *(Turdoides striatus)*

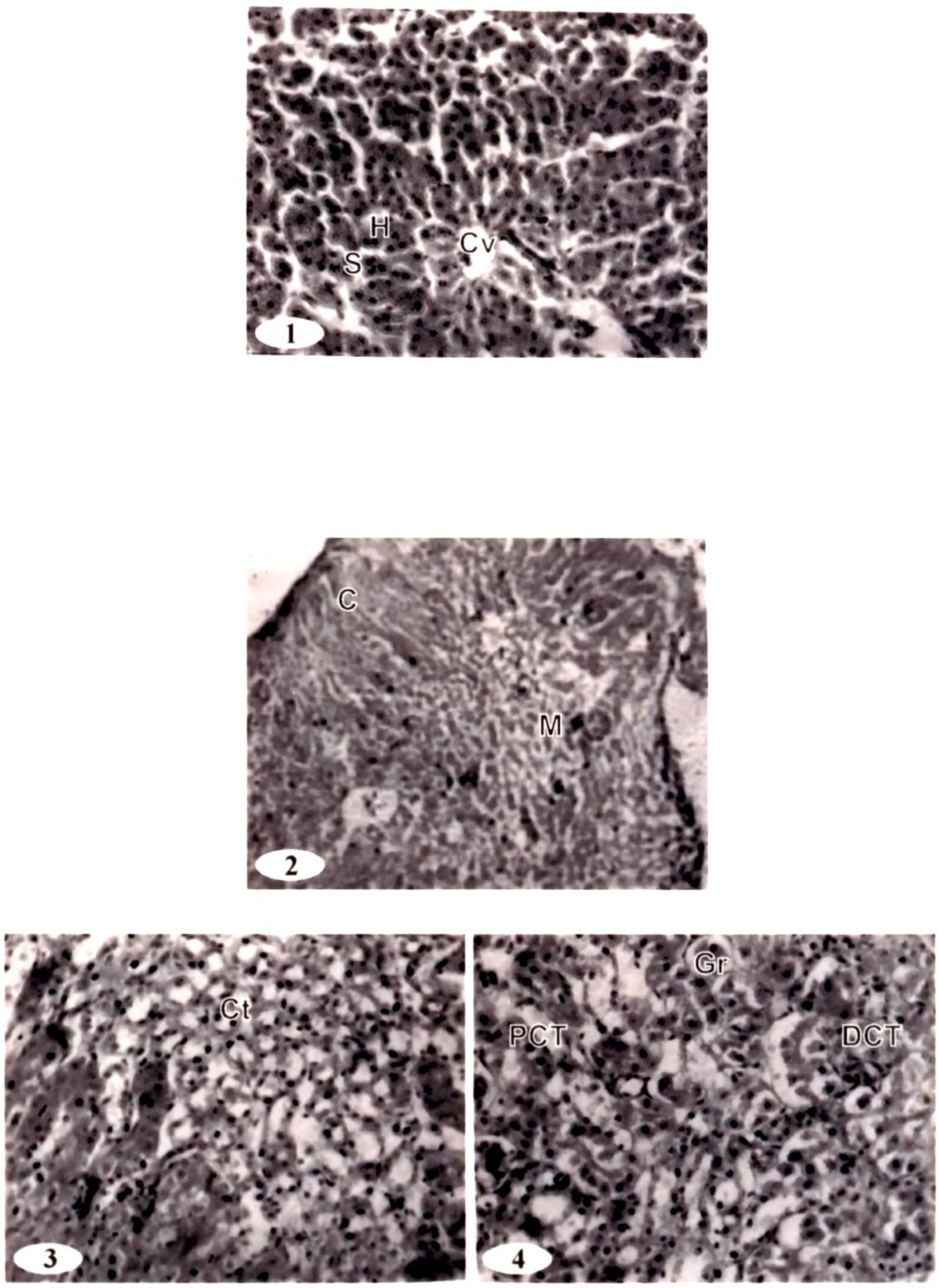
Fig. 2. C: Cortex, **M:** Medullary region (200 X).

Fig. 3. Ct: Collecting tubules (800 X).

Fig. 4. Gr: Glomerular region, **PCT:** Proximal convoluted tubule,

DCT: Distal convoluted tubule (800 X).

Plate III



Explanations to Figures:

Plate IV

Intestine of Jungle Babbler *(Turdoides striatus)*

Fig. 1. T. S. Duodenal part of intestine (200 X).

Fig. 2. Tip of villi (800 X).

Fig. 3. Mid section of Villi (800 X).

Fig. 4. Base of villi (800 X).

V: Villi, **A:** Adventitia and Peritoneum, **LM:** longitudinal muscle, **MM:** Muscularis mucosae, **Bg:** Brunner's glands, **EV:** Epithelium of villus, **CC:** Chief cells, **GC:** Goblet cells, **ECC:** Enterochromaffin cells, **Lu**: Lumen between villi, **C:** Corium of villus, **L:** Lacteal.

Plate IV