POSTNATAL DEVELOPMENT OF THE BURSA OF FABRICIUS IN DUCK

(Anas platyrhynchos)

By

INDU V. RAJ.

THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Anatomy
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA

1999

DECLARATION

I hereby declare that the thesis entitled "**POSTNATAL DEVELOPMENT OF THE BURSA OF FABRICIUS IN DUCK (*Anas platyrhynchos*)**" 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.

Mannuthy
21·08·99

INDU V. RAJ

CERTIFICATE

Certified that the thesis entitled **"POSTNATAL DEVELOPMENT OF THE BURSA OF FABRICIUS IN DUCK (*Anas platyrhynchos*)"** is a record of research work done independently by **Ms. Indu V. Raj,** 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 her.

21/8/99

Dr. Jose John Chungath
(Chairman, Advisory Committee)
Associate Professor
Department of Anatomy
College of Veterinary &
Animal Sciences, Mannuthy

Mannuthy
21.08.99

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Ms. Indu V. Raj**, a candidate for the degree of Master of Veterinary Science in Anatomy, agree that the thesis entitled "**POSTNATAL DEVELOPMENT OF THE BURSA OF FABRICIUS IN DUCK (*Anas platyrhynchos*)**" may be submitted by Ms. Indu V. Raj, in partial fulfilment of the requirement for the degree.

21/8/99

Dr. Jose John Chungath
(Chairman, Advisory Committee)
Associate Professor
Department of Anatomy
College of Veterinary & Animal Sciences, Mannuthy

Dr. K.R. Harshan
Associate Professor and Head
Department of Anatomy
(Member)

Dr. N. Ashok
Assistant Professor
Department of Anatomy
(Member)

Dr. K. Rajankutty
Associate Professor
Department of Surgery
(Member)

External Examiner

ACKNOWLEDGEMENT

With great devotion I would like to express my sincere gratitude and indebtedness to my outstanding mentor, ***Dr. Jose John Chungath****, Associate Professor, Department of Anatomy and Chairman of the Advisory Committee for his expert planning and meticulous guidance. The incessant help rendered by him in all possible ways throughout the period of this study, inspite of his busy schedule, has resulted in this thesis.*

I owe my deep sense of gratitude to ***Dr. K.R. Harshan****, Associate Professor and Head, Department of Anatomy and member of Advisory Committee, for his enduring interest, constructive and erudite suggestions and for placing the resources of the department at my disposition which helped in the successful accomplishment of this work.*

Words fail to express my gratitude to ***Dr. N. Ashok****, Assistant Professor, Department of Anatomy for having given expertise, personal attention and inimitable help as a member of the Advisory Committee during each phase of this research work.*

I am grateful to ***Dr. K. Rajankutty****, Associate Professor, Department of Surgery and member of the Advisory Committee for his timely help and valuable suggestions.*

My sincere thanks are due to ***Dr. C.K. Sreedharan Unni****, Associate Professor, Department of Anatomy for his valuable suggestions. My unreserved gratitude and indebtedness goes to* ***Dr. K.M. Lucy*** *and* ***Dr. S. Maya*** *for their fruitful discussions, sustained encouragement, professional as well as personal guidance and above all for their special love and concern for me.*

I wish to acknowledge Dr. ***Leo Joseph****, Associate Professor, University Poultry Farm, Mannuthy for the sincere help and guidance and for providing the materials necessary for this study.*

I hereby record my sincere gratitude to ***Smt. U. Narayanikutty,*** *Assistant Professor and* ***Mr. K.V. Prasadan,*** *Computer Programmer, Department of Statistics for their help and cooperation in statistical analysis and graph preparation for the thesis.*

I am pleased to express my sincere gratitude to ***Dr. K.M. Ramachandran,*** *Director and Head, Centre of Excellence in Pathology for the facilities rendered during my research.*

I take great pleasure in appreciating ***Dr. K.T. Punnoose,*** *Professor and Head and* ***Dr. M. Mini,*** *Assistant Professor, Department of Microbiology for the facilities provided for this research work. Valuable suggestions given by* ***Dr. V. Jayaprakashan,*** *Associate Professor, Department of Microbiology is gratefully acknowledged.*

Sincere thanks are due to ***Dr. C. Vijayaraghavan,*** *Professor and Head;* ***Dr. S. Geetha,*** *Associate Professor;* ***Dr. Usha Kumari*** *and* ***Dr. Sabiha Hayath Basha,*** *Assistant Professors, Department of Anatomy and* ***Dr. Kumanan,*** *Associate Professor, Department of Biotechnology, Madras Veterinary College for their kind help rendered during my research.*

I am cordially obliged to ***Dr. K.V. Reghunandanan,*** *Associate Professor, Centre for Advanced Studies in Animal Genetics and Breeding for the help rendered in photomicrography.*

I am thankful to ***Dr. G. Raghunathan Nair,*** *Professor;* ***Dr. P.A. Peethambaran,*** *Associate Professor;* ***Dr. P. Anita,*** *Assistant Professor, Centre of Advanced Studies in Poultry Sciences;* ***Dr. N. Vijayan, Dr. N. Divakaran Nair*** *and* ***Dr. Manu Mohan,*** *Assistant Professors, Centre of Excellence in Pathology, and* ***Dr. Sobha Karthiayini,*** *Assistant Professor, Department of Physiology; for their timely help during various stages of the study.*

I sincerely acknowledge the cooperation rendered by the non-teaching staff, Department of Anatomy.

With great pleasure I appreciate the help rendered by ***Mrs. Mini Balram****, Research Associate,* ***Mrs. Mangala****, Research Associate, and* ***Dr. S. Mohan****, Ph.D. Scholar.*

I cherish the spirit of understanding and encouragement rendered to me by my friends, ***Arulmozhi, Chandra, Deepa, Jayasree, Latha, Liz, Marie Sinthiya, Marykutty*** *and* ***Tressy****.*

I express my extreme sense of gratitude to ***Dr. S. Sulochana****, Dean of faculty for providing the necessary facilities to carry out this work.*

I am thankful to ***ICAR, New Delhi*** *for awarding me the fellowship for post graduate study.*

I appreciate ***M/s Peagles****, Mannuthy for the prompt and meticulous typing of the manuscript.*

I am forever fondly beholded to my ***parents*** *and my dear brother* ***Iju****, whose unstinted love and encouragement have always been a perennial source of inspiration to me.*

Above all, I bow to the Almighty for His grace in successful completion of this course.

INDU V. RAJ

To
My Loving Parents and Iju

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Duck husbandry plays a major role in the Indian agricultural economy. The long stretch of coastal areas in Kerala which are waterlogged during a significant period of the year, provide conducive environment for profitable duck farming.

Raising a disease free flock and maintaining a profitable flock depends largely on a strong immune system. Lymphatic system is responsible for the normal functioning of immunity. Functionally, the avian lymphatic system is divided into bursa-dependent and thymus-dependent components. The bursal component is held responsible for humoral immunity and thymic component for cellular immunity (Firth, 1977)

The bursa of Fabricius, named after Hieronymus Fabricius who first discovered the gland in 1621, is a lymphoepithelial gland unique to the class Aves (Adelmann, 1967). It functions centrally as the progenitor of immunocompetent cells, producing antibody molecules, viz., the immunoglobulins.

In order to relate bursal morphology at various ages to the diseases known to affect the bursa Fabricii, it is necessary to have reliable baseline information of the organ in disease-free birds. Since the ducks are considered to be resistant to common avian diseases and are very sturdy in nature, the study of their immune

system histologically and histochemically will be useful for the breeders as well as the researchers in utilising this species profitably and commercially. Moreover, ducks represent the group of waterbirds with lymph-node like organs that are absent in other domestic birds.

Though extensive research has been done on the avian bursa, information regarding the histological and histochemical aspects of this organ in ducks, is very scanty. At this juncture this investigation on the development and regressive changes of the bursa of Fabricius in ducks is an exciting area of research. It is contributory to the existing anatomical knowledge and will be of use for further pathological, physiological and immunological interpretations.

Review of Literature

2. REVIEW OF LITERATURE

2.1 General

According to Chang *et al.* (1957) the primary lymphoid organ, bursa of Fabricius, played a major role in the production of antibodies in young chicken.

The function and morhplogy of the bursa of Fabricius in chicken has been studied extensively (Glick, 1983).

Bursal morphology was studied in Struthioniformes (Forbes, 1877; Van Rautenfeld *et al.*, 1982); Tinamiformes (Forbes, 1877); Falconiformes and Strigiformes (Jolly, 1915; Mathis, 1938); Caprimulgiformes (Jolly, 1915); Cuculiformes (Jolly, 1915; Mathis, 1938; Dominic, 1962); Ciconiiformes and Coraciformes (Dominic, 1962); Pelicaniformes (Ackerman and Knouff, 1964) and Anseriformes (Ward and Middleton, 1971; Sugimura *et al.*, 1975).

2.2 Morphology

The bursa of Fabricius, was reported to be a lymphoepithelial organ peculiar to birds. It appeared as a blind, plicated sac like structure arising as a posterior diverticulum from the proctodeum of cloaca (Payne, 1971; Glick, 1980; Chakravarthy and Sastry, 1982).

The shape of bursa was round or oval in the chicken, but elongated in the Pekin duck (Glick, 1963) and European Starling, *Sturnus vulgaris* (Glick and Olah 1982). The bursa in ducks, *Anas boscas* was described as cylindrical with tapering end (Chakravarthy and Sastry, 1982).

Studies of Ackerman and Knouff (1959) and Hodges (1974) in the bursa of chicken confirmed the occurrence of 11 to 14 numbers of primary plicae and six to seven secondary plicae.

2.2.1 Growth

In chicks the bursa began its development on the tenth day of incubation (Jolly, 1915; Kirkpatrick, 1944a).

At hatching the bursa was well developed and grew rapidly. It attained its full development in the immature bird and got regressed at the onset of sexual maturity (Jolly, 1915; Riddle, 1928). Bradley (1960) and Hinshaw (1953) reported that in chicken the bursa attained its maximum size at four months and then regressed.

Glick (1956) opined that the age at which maximum mean weight was reached varied with the strain of chicken. It was ten to twelve weeks, four to six weeks and eight to eleven weeks, for Barred crosses, White Leghorns and Rhode Island Reds, respectively. Wolfe *et al.* (1962) reported that in White Rock chicken the bursa reached maximum mean weight at ten weeks of age.

Glick (1960) observed that the bursa of the male birds were larger than those of the females during the first four weeks after hatching and later got regressed at sexual maturity. He also noticed that the regression of the bursa corresponded to the enlargement of the testis in male chicken.

According to Ibragimov (1976), in fowl the bursa reached its maximum size at 75 days of age and decreased thereafter. Giurgea (1977) reported that in *Gallus domesticus*, the bursa reached its maximum weight 49 days after hatching. However, according to Romppannen (1982) the bursa grew rapidly from fourth day and reached its maximum size at the sixty third day.

Glick (1956) studied the growth of the bursa of Fabricius in relation to the growth of the body in chicken and found that the highest ratio of bursal weight to body weight was reached at six weeks in Barred crosses, being 0.36 per cent in male and 0.39 per cent in females. In White Leghorns the maximum were 0.52 per cent and 0.50 per cent respectively, at three weeks; whereas in Rhode Islands the highest ratio was 0.37 per cent for both sexes, also at three weeks. Glick (1960) reported that White Leghorn and New Hampshire birds exhibited high positive correlation between the weight of the bursa and body weight up to four weeks of age.

Wolfe *et al.* (1962) observed that the bursal growth rate in White Rock chickens was faster than body growth upto the fourth week of life when the bursa

weighed 0.42 per cent of body weight. Afterwards the rate of bursal growth was lower and at 10 weeks the bursa weighed 0.3 per cent of body weight.

Nair and Sunny (1981) observed that there was a linear regression of the bursa weight on body weight and age of ducks.

In Japanese quails, Yamada *et al.* (1973) noticed five stages in the development and regression of the bursa of Fabricius. The bursa enlarged and attained its mean maximum weight at seven weeks in the males and at six weeks in the females. He further specified that the bursa of quail were more mature at hatching and grew rapidly than that of the fowl.

In Chukar partridge (*Alectoris chukar*) bursal weight peaked at about 10 weeks of age and then decreased. Chukar bursal weights were highly correlated with age and body weight from hatch to 11 weeks of age (Mercer and Woodward, 1987).

In ring necked pheasant (*Phasinus colchicus*), maximum body weight were attained at 26 and 20 weeks of age for males and females, respectively. However, the maximum pheasant bursal weight were observed at 10 and 12 weeks of age in males and females respectively. Afterwards the bursal weight declined abruptly (Mercer and Woodward, 1987).

Glick (1960) reported that in White Pekin ducks, the bursal weight attained its maximum at eight weeks of age, and the mean weight was significantly larger

than the mean bursal weight of chickens at all ages except three and four weeks. King (1975) stated that the greatest development of bursa was observed at approximately three to four months in ducks.

According to Hashimoto and Sugimura (1976) bursa of the ducks grew actively during the first few weeks of post hatching life and reached its maximum mean weight at nine weeks.

2.2.2 Involution

Riddle (1928) stated that in chicken the bursal involution occurred at a time when maximum body weight was almost attained and that bursal involution was usually complete at the time of sexual maturity. After four months of age the bursa underwent regression and nearly disappeared by the end of the first year.

Glick (1956) noticed that the bursa began to atrophy about seven weeks after hatching in White Leghorns whereas in Rhode Island Red atrophy of the bursa began only by thirteenth week.

Wolfe *et al.* (1962) observed that the weight of bursa declined in Barred crosses, White Leghorns and Rhode Island Reds between ten and fourteen, four and seven and eight and thirteen weeks respectively. In White Rocks breed, after 10 weeks there was a decline in bursal weight as the organ began to involute and by 23 weeks of age the bursa was reduced to a remnant.

Warner and Szenberg (1962) stated that the actual time of involution varied among strains and generally it occurred at around two to three months of age in chicken.

Ibragimov (1976) reported that in fowl the bursa decreased in size after 75 days and involution of the organ began at 135 days. According to Giurgea (1977) in chicken the involution of bursa was gradual and by 525 days it was a fluid filled vesicle.

Decrease in bursal weight were observed in White Leghorn chicken between 10 and 16 weeks of age. The involutionary process was almost completed by week 23 when the bursa appeared as fibrotic residue without intact lymphoepithelial structure (Naukkarinen and Sorvari, 1984).

According to Bickford *et al.* (1985) involution was first noted at 24 weeks in single comb White Leghorn chicken during which there was bursal atrophy, variable yellowish discolouration of the mucosa and matting or loss of identity of the mucosal plicae. Involution was completed by 26 weeks and only cicatrized vestiges of bursa were present at 28 weeks.

In Japanese quails, Yamada *et al.* (1977) reported that the bursa regressed very gradually between 18 to 30 weeks of age.

Hashimoto and Sugimura (1976) observed that in White Pekin ducks the bursa, showed a severe regression after 9 weeks of age and at 22 weeks of age, the

bursa remained as a fibrous sack. Chakravarthy and Sastry (1982) stated that in ducks, bursal involution started with the onset of sexual maturity and was completed at the age of 10-12 months, reducing the bursa to a tiny saccule or fibrous cord embedded in the connective tissue and thereafter totally disappeared.

2.3 Morphometry

The earliest bursa weight for a chicken was recorded by Davy (1866). This was a bursa weighing 74 grains at 19 weeks and 6 days.

Hinshaw (1953) and Glick (1956) reported that in young chicken the bursa was at its largest size of 2 to 3 cm long and 1.5 cm wide at four to five months of age.

Wolfe *et al.* (1962) reported that at 1 week of age the bursa had a mean weight of 0.10 ± 0.01 g with a range in size from 0.059 to 0.148 g in White Rocks breed. At 4 weeks, the bursa reached its maximum weight relationship (0.42%) to the body and thereafter grew less rapidly. The bursa reached its maximum mean weight of 3.67 g at 10 weeks of age when it was 0.3 per cent of body weight. At 14 weeks its weight had fallen to 1.41 ± 0.10 g and this was followed by a steady regression and at 23 weeks the weight had reached 0.48 ± 0.09 g.

King and Mclelland (1975) noticed the maximum absolute size of bursa in chickens at 10 weeks of age. It measured 3 x 2 x 1 cm and weighed 4 g.

Ibragimov (1976) reported that in day old chicks the bursa were well developed and weighed about 60 mg. At one month of age bursa had grown nearly 27 times. The bursa reached its maximum size at 75 day of age weighing 3.33 g. Involution started from 135 days and bursa decreased in size after 75 days weighing only 0.12 g at 180 days.

Romppanen (1982) stated that in chicken the bursa measured 138 mg at day 4 and 4416 mg at day 63.

King (1975) reported that in the 6 month old ducks the bursa attained a length of 5 cm and diameter of 7 mm.

Hashimoto and Sugimura (1976) noticed that on the day of hatching the bursa of Fabricius in ducks had a mean weight of 0.08 ± 0.02 (0.13% of the body weight). The maximum absolute mean weight of the bursa was 2.07 ± 0.48 g (0.09% of the body weight) at 9 weeks of age. At 22 weeks the weight reached 0.41 ± 0.43 g (0.02% of the body weight).

The weight of bursa in five weeks old ducks ranged from 0.540 to 1.322 g and at eight weeks it ranged from 0.706 to 1.310 g (Nair, 1990).

2.4 Histology

In chicken histologically the bursa resembled a lymph gland with lymphoid follicles and was lined by pseudostratified columnar epithelium (Calhoun, 1954).

The wall of the definitive bursa was reported to be divided into three tunics - the outer tunic the serosa, the middle was the muscular tunic made of two layers and the inner tunic was the mucosa lined by the surface epithelium of pseudostratified columnar type in chicken (Payne, 1971; Hodges, 1974; Firth, 1977) and in quails (Basha, 1993)

The external serosa of the bursa of chicken covered a muscularis composed of variable muscle layers. Normally at least two layers were seen running obliquely or at right angles to each other. The main branches of the blood vessels supplying the organ lay between the muscle layers and branches of these vessels extended up through the corium of each plica. Also passing part of the way up the corium were muscle fibres from the innermost muscle layer (Hodges, 1974; Firth, 1977).

According to Malewitz and Calhoun (1958) in turkey, the tunica muscularis of bursa consisted of outer circular and inner longitudinal layer of smooth muscle fibres. Similar reports were made by Basha (1993) in quail.

Hashimoto and Sugimura (1976) reported that in White Pekin ducks, the outermost part of bursa in cross section consisted of a circular strand of smooth muscle and serosa.

The bursal mucosa consisted of a lining epithelium and lamina propria filled with lymphoid follicles in all avian species (Hodges, 1974; Firth, 1977;

Glick, 1983; Basha, 1993) including ducks (Hashimoto and Sugimura, 1976; Chakravarthy and Sastry, 1982).

The bursal mucosa was thrown into folds, the primary plicae each of which branched to form secondary plicae in chicken (Hodges, 1974; Glick, 1983; Thandavamoorthy, 1989) in quails (Basha, 1993) and in ducks (Chakravarthy and Sastry, 1982). In the European starling plicae were absent (Glick and Olah, 1982).

In chickens (Payne, 1971; Thandavamoorthy, 1989) and quails (Yamada *et al*., 1977; Basha, 1993) the plicae increased in height and diameter concommitant to the growth of lymphoid follicles upto the age of sexual maturity.

2.4.1 Epithelium

The surface epithelium which lined the inner faces of the plicae, was basically of tall columnar, pseudostratified type in chicken (Ackerman and Knouff, 1959; Payne, 1971; Hodges, 1974; Glick, 1983).

In turkey (Malewitz and Calhoun, 1958) and in quails (Basha, 1993) the lining epithelium was pseudostratified columnar at the plical tip and at certain folds between the plica it was simple columnar.

Chakravarthy and Sastry (1982) reported that the lining epithelium of the bursa was columnar in ducks.

Ackerman and Knouff (1959); Hodges (1974); Thandavamoorthy (1989) recorded three cell types in the bursal epithelium of one day old chick. Type I, an oval cell with round to oval nucleus and clear cytoplasm containing a number of saliva-resistant, PAS positive and sudanophilic granules. The nucleus was round to oval but sometimes irregular. The Type II, the most numerous cells appeared as columnar cells with an oval nucleus. Where the apex of each follicle became continuous with the surface epithelium, there was a group of cells known as the epithelial tuft. These cells differed from surrounding epithelium and were of low columnar type with an oval nucleus above the midpoint of the cell. Type III was a narrow goblet cell containing a hyperchromatic nucleus and PAS-positive secretory material. Similar type of cells were recorded by Basha (1993) in quail.

The epithelium covering the plicae could be recognised into follicle associated epithelium (FAE), covering the follicle which occupied 10 per cent of the bursal surface and that located between the follicles, the interfollicular epithelium (IFE) which covered the remaining 90 per cent of the bursal surface (Naukkarinen *et al.*, 1982; Olah and Glick, 1992; Sanjayan *et al.*, 1996). In chicken (Thandavamoorthy, 1989) and in quails (Basha, 1993) the follicle-associated epithelial cells were found to be pale columnar cells which was distinguishable from the darkly stained interfollicular epithelial cells.

The FAE was supported by three to five layers of stratified epithelial cells, extension of the corticomedullary epithelial cells. The FAE, consisted of M cells

and scattered secretory dendritic cells. Lamellated epithelial bodies similar to Hassal's' bodies were formed by infoldings of the supporting layers of the FAE into the medulla. The lamellated bodies enclosed secretory dendritic cells but not lymphocytes. The infoldings varied from follicle to follicle and number of follicles increased with age (Olah and Glick, 1992).

Sugimura *et al.* (1975) and Hashimoto and Sugimura (1976) observed that in White Pekin ducks the epithelium coating the inner surface of the bursa was divided into two parts, the IFE and FAE. The IFE was pseudo-stratified columnar and the epithelial cells were mucous cells with secretory material. On the other hand, the FAE was stratified cuboidal or columnar in shape which extended into medulla.

2.4.2 Lymphoid follicle

The bursal mucosa in chicken consisted of a connective tissue frame work enclosing many lymphoid follicles. The connective tissue consisted of a network of fine collagen fibers with numerous reticular fibres surrounding the follicles (Hodges, 1974; Firth, 1977). Similar reports were made by Basha (1993) in quails.

The follicles were spherical in the developing chick but later, with the proliferation of the round cells and constant increase in size, they assumed a polyhedral outline (Bradley, 1960).

Each follicle was divisible into two basic parts - cortex and medulla in all birds (Ackerman and Knouff, 1959; Hodges, 1974; King and McLelland, 1981; Hashimoto and Sugimura, 1976; Olah and Glick, 1978: Thandavamoorthy, 1989; Basha, 1993; Sanjayan *et al.*, 1996; Tizard, 1996).

A layer of epithelial cells with distinct basement membrane separated the cortex and medulla. This undifferentiated epithelial cell layer was continuous with the surface epithelium of the plica in chicken (Payne, 1971).

In the bursa of chicken Frazier (1974) recorded reticulo epithelial cells to be rare in the cortex and common in the medulla where they were in close association with the basement membrane and formed a supporting network for the lymphoid cells.

The cellular content of the cortex in the bursa of chicken consisted mainly of closely packed small lymphocytes and lymphoblasts, mitotic figures and macrophages (Thorbecke *et al.*, 1957; Frazier, 1974; Tizard, 1996). The blood supply to the cortex was not well developed and the capillaries were in close association with the basement membrane (Frazier, 1974). Beneath the capillaries the basement membrane separated the cortex and the medulla. Adjacent to the basement membrane the outermost layer of the medulla comprised of a single layer of undifferentiated epithelial cells in continuity with the surface epithelium of the plica (Hodges, 1974; Glick, 1983).

The cellular content of medulla consisted of macrophages (Frazier, 1974), plasma cells, numerous lymphocytes and lymphoblasts in the bursa of chicken (Olah *et al.*, 1979).

Naukkarinen and Sorvari (1984) reported that the cellular population of the medulla of the bursal follicle included B lymphocytes, T lymphocytes, lymphoblast, plasma cells, dendritic reticulum cells and histiocytic reticulum cells. The ultrastructure and histochemical character of these cells were typical of the germinal centres of the lymphnode. An epithelial structure identical to thymic Hassall's corpuscle was present in the medulla of the bursal follicle.

Plasma cells could be commonly located in the interfollicular tissue and beneath the bursal epithelium of chicken (Clawson *et al.*, 1967) and ducks (Hashimoto and Sugimura, 1976). At 20-30 days of age maximum number of plasma cells were recorded in the bursa of ducks (Hashimoto and Sugimura, 1976) and quails (Basha, 1993).

In chicken the epithelial arches of corticomedullary border, consisted of small and medium lymphocytes (Olah *et al.*, 1979; Glick and Olah, 1982) and immature secretory cells, blast cells and mitotic figures were present (Olah and Glick, 1987).

In chicken the lymphocyte of both cortex and medulla of bursal follicles were similar in size (4-8 μm) and ultrastructure (Clawson *et al.*, 1967).

Olah and Glick (1978) measured the average number of follicles per plica and found it to be 820 and the total number of follicles in the bursa of a 4 week old chicken as 8000-12000.

In the bursa of the duck, the plicae contained several lymphoid follicles surrounded by connective tissue trabeculae (Hashimoto and Sugimura, 1976).

Each follicle contained large lymphocytes sparsely distributed in the central region and surrounded peripherally by closely packed small lymphocytes (Chakravarthy and Sastry, 1982). The cortex contained large and medium sized lymphocytes while medulla contained small lymphocytes (Sugimura *et al.*, 1975).

2.4.3 Development of follicles

Rodak and Krejci (1966) observed that in day old chicks the follicles of bursa consisted of medullary cells of epithelial origin with only scattered lymphocytes and lymphoblasts. The cortical part showed only a negligible development. In five to nine day old chicks characteristic changes of follicles occurred in the medulla with a marked increase in pyroninophilia of medullary cells which assumed the character of lymphoblasts.

Yamada (1966) reported that in chicken during the early stages of growth, histologically the medulla was partially or completely enveloped with cortex and follicles enlarged gradually. During the middle and last stage of growth the follicles enlarged still more with a waved basement membrane.

Ibragimov (1976) reported that at 75 days of age when bursa reached its maximum size, the lymphatic follicles began to form with distinct cortical and medullary layers in chicken. The maximum follicle diameter was recorded at day 65 in chicken.

During the mature bursal stage, the most voluminous bursal tissue compartment was of lymphatic tissue (lymphoid follicles). It increased relatively from 62 per cent to 86 per cent and absolutely from 85 mg to 3810 mg during the period from fourth to sixteenth day of age. The follicular diameter increased from 185 to 720 μm. The follicular cortex was thin initially and its relative amount was 18% which later increased and reached the level of follicular medulla. The relative volume of FAE decreased from 3.6 per cent at day 4 to 0.4 per cent at day 84 (Romppanen, 1982).

According to Thandavamoorthy (1989) a well defined cortex could be identified only at 15 days posthatch in chicken.

Histological studies conducted on Japanese quail by Yamada *et al.* (1977) indicated that during the first 5 weeks of life, lymphoid follicles and plicae enlarged. By fourth week, connective tissue hyperplasia and fusion of the plicae, leading to gradual occlusion of the bursal lumen and reduction in follicle size were noticed.

Basha (1993) reported that the cortex and medulla were clearly defined only by 15 days of age in quails.

In the day old ducklings, the lymphoid follicles contained well differentiated cortex and medulla. The number of bursal lymphoid follicles was almost fixed from the day of hatch upto 13 weeks. The diameter of follicles increased upto ninth week and decreased thereafter. Thus, the size of follicles changed with age and coincided with the weight of the bursa (Hashimoto and Sugimura, 1976).

2.4.4 Involution

During regression of bursa in chicken, lymphocytes were lost from the cortex and medulla and the epithelial component of the medulla became more prominent. The epithelial reticulum took to metaplasia and gave rise to acinar structures lined by columnar or pseudostratified epithelium. The bursa became fibrous and diminished greatly in size (Jolly, 1915).

Yamada (1966) observed that in chicken during the early stages of involution the subepithelial connective tissue proliferated over all portions. During the middle stage of involution accretion of plicae occurred form the basal portion. Further, during the last stage of involution there was advancement of accretions and cyst formation and all follicles lost their normal structures and the bursa became fibrous.

Glick (1983) noted that during the period of bursal regression, a distinct separation occurred between the FAE and IFE with an increase in convolution of the IFE in the bursa of chicken.

In chicken Naukkarinen and Sorvari (1984) recorded that during the seventeenth week, the FAE lost its endocytic capability. The mucin droplets appeared in the follicular medulla initiating the large mucoid cysts that were seen in the later phases of involution. The process of involution was almost completed by the 23rd week when the bursa appeared as fibrotic residue without intact lymphoepithelial structure.

Histological characteristics of involution in chicken were summarised by Bickford *et al.* (1985). They noticed atrophy and exfoliation of plical epithelium, subepithelial stromal fibrosis and ultimate collapse of plicae. They further specified that there was liquefactive necrosis of first medullary then cortical elements of follicles, which seemed to progress from basal to apical portion of the plicae, progressive proliferation of stromal connective tissue and infiltration of macrophages into areas occupied by necrotic follicles. Finally complete fibrosis and contraction of muscularis that surrounded the cicatrized remains of the mucosa preceeded the formation of a firm nodule like structure.

In chicken, Milicevic *et al.* (1986) reported that during regression numerical density, surface density and diameter of FAE decreased and there was reduction of volume and density of follicular cortex in bursal mass. There was

increase in the volume density of stroma. The damage to bursal epithelium was the earliest sign of involution and occurred before the alteration of the follicular structure.

Histological studies conducted on Japanese quail by Yamada *et al.* (1977) indicated that between 18-30 weeks of age regression was completed. There were no normal lymphoid follicles and the fibrous bursae were replaced with adipose tissue.

In White Pekin ducks, according to Hashimoto and Sugimura (1976) after 13 weeks of age there was a sharp decrease in the number of lymphoid follicles in the bursa. At 22 weeks of age the bursa contained only a few lymphoid follicles which consisted mainly of hyperplastic smooth muscle and adipose tissue.

Scala *et al.* (1988) observed an increase in the interfollicular connective tissue, reduction in the lymphocyte population and loss of cells through the wide ducts which directly opened into the bursal lumen as the involutionary change in 100 day-old ducks. By 150 days of age the bursal follicles disintegrated but cysts were not observed in the involuted bursa of ducks.

2.5 Histochemistry

The histochemistry of the developing bursa was described by Ackerman and Knouff (1959; 1964). The results indicated high metabolic activity. Mesenchymal cells adjacent to the developing follicles of the bursa showed

intense nuclear and cytoplasmic alkaline phosphatase activity. Epithelial cells and lymphocytes exhibited only moderate nuclear activity. However the cells of the epithelial tuft (FAE) had intense alkaline phosphatase activity between the second and eighth days post hatching (Ackerman and Knouff, 1959).

Fennel and Pearse (1961) observed isocitrate, malate and succinic dehydrogenase activity in the bursa of Fabricius in chicken. Acid phosphatase activity was more intense in the epithelium of the bursa of chicken than alkaline phosphatase activity.

Rodak and Krejci (1966) reported that there was a marked increase in pyroninophilia of medullary cells which assumed the character of lymphoblasts and showed high alkaline phosphatase activity and decreased mitotic activity.

According to Basha (1993) the bursal epithelium revealed intense acid phosphatase activity and moderate alkaline phosphatase activity in the quail of all age groups.

Ackerman and Knouff (1959), Hodges (1974) and Thandavamoorthy (1989) opined that the bursal epithelium exhibited positive reaction to PAS reagent in chickens. Basha (1993) made similar observations in quails of all age groups.

Basha (1993) reported lipid substances surrounding the degenerating follicles of the involuted bursa.

Materials and Methods

3. MATERIALS AND METHODS

In the present experiment, the bursa of Fabricius collected from 51 apparently healthy ducks of various age groups were studied for their morphological and histological development during the postnatal life of 155 days. Ducklings of the same hatch were procured from the University Poultry Farm, Mannuthy and reared under intensive system of management. The ducklings were not given any vaccination. Feed and water were provided *ad lib*.

The study was carried out in birds of different ages, ranging from day-old to 155 days as shown in Table 1. From the day of hatch upto 20 days the bursa were collected at five days interval, from 20 to 65 days at weekly interval and 65 to 155 days at 15 days interval.

The bursa was collected from each bird by surgical method of bursectomy described by Chang *et al.* (1957). Pressure was applied to the duckling's back with the left hand and an incision was made with a scalpel blade at the base of the tail, just above the upper lip of the vent. The bursa was then grasped with curved forceps at the anterior end and eased towards the opening. It was then excised very close to its attachment to the cloaca.

3.1 Morphological study

The body weight of the birds were recorded. The bursa collected surgically was washed in normal saline and mopped with blotting paper and examined for the gross appearance, colour and shape. Afterwards all the biometric observations like length, diameter (top and bottom) and weight were recorded. The anatomical position and relationships of bursa of Fabricius was studied in the culled birds bought from the University Poultry Farm, Mannuthy.

3.2 Histological study

The same tissue collected for morphological observation was used for microscopic studies.

Each bursa 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. Zenker's fluid
3. Bouin's fluid
4. Chilled acetone (4°C)
5. 90 per cent alcohol

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.

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. Gomori's aldehyde fuchsin technique for elastic fibres (Drury and Wallington, 1980).

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

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

To study the details of different cell types of the bursa of Fabricius, the following staining techniques were adopted.

1. Methyl green-pyronin method for the demonstration of plasma cells (Singh and Sulochana, 1996).

2. Toulidine Blue method (Singh and Sulochana, 1996) for the demonstration of the mast cells.

For the histochemical studies, the following methods were employed.

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

2. Oil red "O" in propylene glycol method for lipids (Luna, 1968)

3. Modified Gomoris' method for alkaline phosphatase activity (Pearse, 1977)

4 Naphthol AS-B_1 phosphate method for acid phosphatase (Barka, 1960).

3.3 Immunohistochemistry

Immunoperoxidase staining was conducted as per Islam *et al.* (1993) with slight modifications. The impression smears were fixed in methanol for 15 minutes and the smears were treated with 3 per cent BSA (Bovine serum albumin) at 37°C for 30 minutes to block the nonspecific binding sites of tissues. Slides were washed thoroughly in phosphate buffer saline and placed in a methanol bath containing 3 per cent hydrogen peroxide and incubated at room temperature for 30 minutes to inhibit the endogenous peroxidase. Subsequently the slides were washed in phosphate buffer saline and a predetermined working dilution (1:500) of HRPO conjugate (conjugated with antichicken IgG, Sigma, USA) was added

over the slides and incubated at 37°C for 1 hour. Then slides were washed thoroughly in phosphate buffer saline and treated with 0.05 per cent DAB (Diamino Benzadine, Sigma) in phosphate buffer saline containing 0.05 per cent freshly added hydrogen peroxide. Slides were incubated at room temperature for 10 minutes in a dark place. The sections were examined under light microscope and the peroxidase activity was obtained as dark brown precipitate.

3.4 Micrometry

Micrometry was done using an occulometer to record the following.

1. The extent of the cortex and the medulla in the bursal follicles in different age groups.
2. Total number of the bursal follicles per cross section.
3. The height and the width of the plicae.
4. The diameter of the follicle and the size of the bursal follicle in different age groups.
5. The cellular differentiation and lymphocyte population in unit area of bursal tissue.

3.5 Statistical analysis

Data collected were analysed as per the procedures of Snedecor and Cochran (1967). A multiple linear regression of the bursa weight on the body weight and age (in days) was fitted to the data. Simple as well as partial significant coefficients between the different factors were calculated and tested for significance.

Results

4. RESULTS

4.1 Morphology

4.1.1. Body weight

The mean body weight of the White Pekin ducks at different ages are presented in Table 2. On the day of hatch the body weight was 31.66 ± 0.45 g which increased gradually to 180 ± 1.54 g by 20 days of age. Thereafter a spurt in growth was noticed and the birds weighed 2206.6 ± 1.86 g at 155 days of age (Fig.5).

4.1.2. Growth of the bursa of Fabricius

In day old ducklings the bursa of Fabricius could be seen as an elongated blind sac like structure with a tapering apex and smooth pale yellow outer surface (Fig.3). The bursa was connected to the dorsal proctodeal wall of the cloaca by a short stalk and its lumen opened into the cloaca (Fig.2 and 16).

In all the young ones, up to 20 days the bursa was pale yellow in colour, cylindrical and caecum like with a tapering apex. Thereafter, the bursa increased in both length and diameter and by 58 days it was more cylindrical with a pointed apex and yellowish white colour (Fig.3). Thereafter by 155 days, the bursa remained cylindrical and its height and diameter were greatly reduced. The colour of the 155 day old bursa was pale white.

The inner luminal surface of the bursa showed about six small folds in day old ducklings. By 10 days, the number of plicae increased to eight and were very prominent. There were two large well developed longitudinal folds on the ventral aspect and about six smaller folds all round the circumference inside the bursal lumen. The number of primary plicae increased to eleven by 28 days of age (Fig.1), and thereafter by 95 days the number of plicae decreased to eight. The bursa of 155 day old birds had only two prominent longitudinal plicae on its base and four very small indistinguishable folds all round the circumference.

The size of the lumen was smaller in the newly hatched ducklings but it increased as age advanced.

4.1.2.1. Weight of the bursa of Fabricius

The overall weight of the bursa of Fabricius of White Pekin ducks during post hatch life is shown in table 3. Table 4 records the bursa-body weight relationship at different age groups.

On the day of hatch the bursa had a mean weight of 0.07 ± 0.45 g. This mean weight represented 0.23 per cent of the gross body weight. After five days there was over a 100 per cent increase in the mean weight and similar increases occurred during the next five days. The bursa reached its maximum weight relationship (0.49 %) to the body at five days of age and thereafter grew less rapidly than the body.

The bursa of ducks showed the most rapid growth in the first 28 days after hatch and reached its maximum mean weight of 2.22 ± 0.01g at 58 days of age. The mean bursal weight at this age represented 0.17 per cent of body weight. During the next week there was a 30 per cent decrease in mean weight of the bursa to 1.60 ± 0.01 g. A small increase in the mean weight occurred between day 65 and day 95 but this was followed by a steady regression and at 155 days the weight had reduced to 1.20 ± 0.05 g. The mean bursal weight at this age represented only 0.05 per cent of the body weight (Table 3 and 4) (Fig.4, 6 and 7).

4.1.2.2. Length and diameter of the bursa of Fabricius

Table 5 records the length and diameter of bursa at different ages.

In the day old birds the bursa had an average length of 11.33 ± 0.570 mm. There was a continuous increase in its average length up to 58 days post hatch, but following this it showed a decreasing trend and at 155 days of age the bursa measured 25.67 ± 1.45 mm (Fig.3 and 9).

The bursa was wider at its bottom than at the apex. Bursa of day old birds showed a mean diameter of 1.33 ± 0.16 mm and 2.33 ± 0.62 mm at its apex and base respectively. By five days of age the bursa showed two fold increase in its diameter at both the ends. Thereafter the diameter increased gradually and reached its maximum size of 7.66 ± 0.33 mm and 11.33 ± 0. 67 mm at its apex and base portions respectively, at 58 days of age. There was a considerable

decrease in the overall diameter of the organ between 65 and 155 days of post natal period (Table 5).

4.1.2.3. Statistical Analyses

Table 6 records the statistical analyses.

The linear regression of the weight of bursa on the body weight and age of the bird was found to be highly significant ($P<0.01$). It was observed that 72.2 % of the total variation in weight of bursa was explained by the variation in the age and body weight of the bird estimating a multiple coefficient correlation of 0.850. On assessing the simple pairwise correlation coefficient (Table 6) highly significant positive correlation was observed between age and body weight ($r=0.968$);bursa weight and body weight ($r=0.684$); bursa weight and its length ($r=0.963$). Significant positive correlation coefficient was observed between the weight of bursa and the age as well as the weight of bursa and its diameter (top and bottom) ($r=0.567$ and $r=0.586$). A negative correlation was observed between age and per cent weight of bursa (to body weight) ($r=-0.841$).

The partial correlation coefficient between weight of bursa and age of bird keeping body weight constant was -0.69 ($P<0.01$); where as that between bursa weight and body weight when age of bird was held constant was 0.788 ($P<0.01$). Thus when the body weight of the bird was kept constant, 47.6 per cent of total variation in the weight of the bursa was accounted for by age and when age of the

bird was kept constant 62.4 per cent of the variation in the weight of the bursa was accounted for by body weight.

4.2 Microscopic observations

In birds of all ages the wall of the bursa consisted of three tunics - the outermost tunica serosa, the middle tunica muscularis and the innermost tunica mucosa.

The tunica serosa enveloped the entire organ. It was very thin in the day old birds. As age advanced, the bursal serosa increased in thickness gradually (Fig.12).

The tunica muscularis consisted of an outer circular and inner longitudinal layer of smooth muscle fibres (Fig.12). In the day old birds the inner longitudinal layer of smooth muscle fibres could not be delineated clearly and the outer layer of circular smooth muscle was thin. In the 30 days old bursa the inner longitudinal and outer circular layers could be clearly differentiated. The longitudinal layer was not complete all around and was seen only at the base of the plica. Thereafter, with advancement of age both the muscle layers increased in thickness gradually. Blood vessels were found between the muscle layers, in birds of all ages. The branches of these vessels passed up through the corium of each plica.

The innermost, tunica mucosa consisted of a lining epithelium and lamina propria filled with lymphoid follicles in birds of all ages (Fig.10).

The mucosa was arranged in folds. The primary plicae were subdivided into secondary plicae. Table 7 records the changes in the number and size of the bursal plicae at different ages. In the day-old birds the average length and width of the two large ventral plicae were 1575 µm and 525 µm respectively. The small plicae all round the circumference of bursal lumen had an average length of 350 µm and width of 507.5 µm. in the day old bursa. A gradual increase in size of both the large and small plicae could be recorded up to 58 days of age when the average length and width of the large plicae were 4462.5 µm and 1662.5 µm respectively and those of the small plicae were 1600 µm and 1365 µm respectively. There after the size of the plicae showed a reducing trend and at 155 days, the average length and width of the large plicae were 3200.25 µm and 980.5µm respectively. The average length of the small plicae was reduced to 940 µm and width was only 82[illegible] µm. The bursa of 140 and 155 days old ducks showed fusion and ultimate collapse of the plicae (Fig.25).

The lining epithelium of the bursal mucosa was of pseudostratified columnar type in all age groups (Fig.11). The average height of the columnar cells increased up to 35 days of age and remained constant thereafter. By the 95th day bursa showed thinning and vacuolation of plical and interplical epithelium (Fig.29). Thereafter till 155 days of age, pit formation and other severe

degenerative changes were noticed in the epithelium lining the bursal mucosa. Epithelial fusion was evident from 140 days of age (Fig.30).

Upto 95 days of age, three types of cells could be identified in the lining epithelium of bursal mucosa. Type I cell was columnar in shape with a round or oval nucleus. Type II cell, was columnar with an oval nucleus located below the midline of the cell. Where the apex of each follicle became continuous with the surface epithelium the Type II cell was of low columnar type with on oval nucleus located above the midpoint of the cells. The Type III, was a goblet cell found among the columnar cells.

The epithelium was distinguished into two alternating areas, the follicle-associated epithelium (FAE) or the epithelial tuft showing pale columnar cells at the tip of the plicae and the inter-follicular epithelium (IFE) showing dark columnar cells. In the IFE cells mild degenerative changes were recorded from 42 days of age. But there was no change in the FAE cells until 95 days of age. Thereafter detachment of epithelial cells from the underlying follicles resulted in spillage of lymphocytes into the lumen from the degenerating follicles.

The interfollicular and subepithelial connective tissue were made up of a network of fine collagen fibres (Fig.15), with numerous reticular fibres (Fig.32) and few elastic fibres (Fig.33), in birds of all ages. In the 145 days old bursa, proliferation of the connective tissues were evident (Fig.27). A tremendous increase in thickness and density of the interfollicular connective tissue and

subepithelial stromal fibrosis were noticed at 155 days of age. Large blood vessels were found in the interfollicular connective tissue and at the base or centre of each plica (Fig.13).

Eosinophils and plasma cells were noticed in the interfollicular and subepithelial connective tissue. Plasma cells appeared on the day of hatch itself and their number increased markedly during 20 to 28 days of post-hatch period and thereafter a decrease was observed. Macrophages were present in the propria closely associated with the lymphoblasts of the follicles. Mast cells were also observed in the lamina propria.

The lymphoid follicles present in the lamina propria were spherical in the day old duckling but later by 20 days, they assumed a polygonal and elongated outline. The number of bursal lymphoid follicles during post natal life showed almost no changes up to 65 days of age. Thereafter, a gradual decrease was observed upto 125 days post hatch. At 155 days, a 50 per cent reduction in the number of follicles was observed and only 60 follicles could be counted per cross section in the lamina propria of the bursal mucosa (Table 7) (Fig.23).

Few microscopic alterations other than progressive expansion of follicles were observed up to 58 days of age. The average diameter of the lymphoid follicles in the bursa of day old ducklings was 162.75 μm and by 58 days a maximum average diameter of 621.25 μm was recorded. Later on, a gradual decrease in the size of follicles was noticed up to 145 days post hatch. Widely

scattered and isolated atropied follicles were recorded at 155 days. At this age a 58 per cent drop in size of the follicles was noticed with an average follicular diameter of 180.12 μm (Table.7) Finally, the mucosal remnants consisted of numerous coalescing degenerating follicles intermingled with infiltrating heterophils, macrophages and fibrous connective tissue (Fig.28).

In birds of all age groups each follicle consisted of an outer thin cortex and inner large medulla separated by a layer of undifferentiated epithelial cells with distinct basement membrane and a thin layer of capillary (Fig.10 and 14). This undifferentiated epithelial layer with basement membrane was in continuity with the basement membrane and surface epithelium of the plica.

Both the cortex and the medulla possessed a supporting network of stellate reticuloepithelial cells whose meshes were filled with lymphoid cells in birds of all age groups. In the cortex, the network of reticuloepithelial cells were continuous with the surrounding connective tissue. The undifferentiated epithelial cells between the cortex and medulla were of low cuboidal type with pale staining, round nuclei in all the birds.

The cortex was more deeply staining than the medulla and contained densely packed large and small lymphocytes with few macrophages, plasma cells and many mitotic figures in the bursa of day old ducklings. Plasma cells were recorded in the cortex upto 28 days of age (Fig.31). Large lymphocytes predominated in the cortex of the lymphoid follicles up to 58 days of age. Later

on, small lymphocytes predominated over large lymphocytes. In the bursa of 140 and 155 days old ducks only small lymphocytes could be detected in the folliclular cortex.

In the medulla large lymphocytes were noticed near the corticomedullary junction and loosely arranged small lymphocytes occupied the central region of the lymphoid follicles up to 125 day of age. In the 140 and 155 days old bursa only small lymphocytes could be identified in the follicular medulla. Loosely arranged reticuloepithelial cells and few macrophages could be detected in the medulla of the lymphoid follicles in birds of all age groups. The medulla was devoid of blood capillaries in all the birds.

The number of lymphocytes in each follicle increased gradually up to 58 days of age (Fig.18, 19 and 24). In the bursa of day old ducklings an average of 890 lymphocytes could be counted per follicle (Fig.17). Though both large and small lymphocytes were detected, former were predominant. A maximum number of 4,500 lymphocytes could be counted in each follicle and the majority were large lymphocytes. There after the lymphocyte population dropped gradually and after 140 days a 70 per cent decrease was recorded with an average of only 860 small lymphocytes in each follicle (Fig.20, 21 and 24). There was severe depletion of cortical lymphocytes in the 140 and 155 days old bursae (Table 7) (Fig.22).

Changes in the width of cortex, diameter of medulla and the number of lymphocytes in lymphoid follicles are tabulated in Table 7. In the day-old birds

the cortical width was 43.75 μm and medullary diameter was 70μm. A progressive expansion in the width of cortex and medullary diameter were observed upto 58 days of age when they measured 110.58 μm and 408.33 μm respectively. Thereafter, both the parameters decreased gradually up to 140 days of age. Liquefactive necrosis of medullary and cortical tissue of the follicle and interstitial oedema were noted from 110 days of age (Fig.26). In the 155 days old bursa, after a 60 per cent reduction in cortical width and 50 per cent reduction in medullary diameter they measured 16.05 μm and 159.5μm respectively (Table 7).

4.3 Histochemical observations

The lining epithelial cells of the bursal mucosa showed a positive PAS reaction in birds of all ages (Fig.34 and 35).

The epithelial cells of the bursal mucosa showed metachromasia with toulidine blue in ducks of all ages (Fig.36).

Lipid droplets were localized in the lamina propria of immature bursa while in the involuted bursa adipose tissue surrounded the degenerating follicles (Fig.39).

Intense acid phosphatase activity was recorded in the lining epithelium of the bursal mucosa. Moderate acid phosphatase activity was detected in the lymphoid follicles. Mild alkaline phosphatase reaction was observed in the bursal mucosa in all the age groups studied (Fig.37 and 38).

4.4 Immunohistochemistry

The positive immunoperoxidase staining, indicated the presence of antibodies in the bursa of Fabricius. The positive staining was characterised by the deposition of dark brown granular precipitates at the site of antibody localisation. The maximum positive reaction was recorded from 35 to 58 days of age (Fig.40).

Table 1. Age and number of birds used for the experiment

Group Number	Age of birds (in days)	Number of birds
1	0	3
2	5	3
3	10	3
4	15	3
5	20	3
6	28	3
7	35	3
8	42	3
9	50	3
10	58	3
11	65	3
12	80	3
13	95	3
14	110	3
15	125	3
16	140	3
17	155	3
	Total	51

Table 2. Age and body weight of White Pekin ducks (Mean ± S.E.)

Age in days	Body weight (g)
Day old	31.66 ± 0.45
5	36.66 ± 3.33
10	95.00 ± 2.88
15	125.00 ± 2.88
20	180.00 ± 1.54
28	506.66 ± 0.66
35	520.00 ± 1.54
42	793.33 ± 3.59
50	1100.00 ± 4.75
58	1326.67 ± 0.66
65	1406.60 ± 2.95
80	1633.33 ± 2.40
95	1833.33 ± 4.05
110	1856.66 ± 4.59
125	2166.66 ± 2.11
140	2100.00 ± 0.24
155	2206.66 ± 1.86

Table 3. Weight of the bursa of Fabricius of White Pekin ducks at different ages (Mean ± S.E.)

Age in days	Weight of bursa (g)
Day old	0.07 ± 0.45
5	0.18 ± 0.01
10	0.41 ± 1.00
15	0.45 ± 0.02
20	0.60 ± 0.01
28	1.12 ± 0.07
35	1.35 ± 0.05
42	1.36 ± 0.08
50	2.03 ± 0.03
58	2.22 ± 0.01
65	1.60 ± 0.01
80	1.65 ± 0.02
95	1.68 ± 0.02
110	1.63 ± 0.02
125	1.28 ± 0.27
140	1.27 ± 0.04
155	1.20 ± 0.05

Table 4. Per cent weight of bursa (to body weight) at different ages in White Pekin ducks

Age in days	Percentage weight of bursa to body weight
Day old	0.23
5	0.49
10	0.43
15	0.36
20	0.33
28	0.22
35	0.26
42	0.17
50	0.18
58	0.17
65	0.11
80	0.10
95	0.09
110	0.09
125	0.06
140	0.06
155	0.05

Table 5. Length and diameter of bursa at different ages (Mean ± S.E)

Age in days	Length (mm)	Diameter (mm)	
		Top	Bottom
Day-old	11.33 ± 0.57	1.33 ± 0.16	2.33 ± 0.62
5	14.00 ± 1.00	3.33 ± 0.33	5.00 ± 0.58
10	16.00 ± 0.29	3.53 ± 0.33	5.00 ± 0.01
15	16.68 ± 0.67	3.67 ± 0.29	5.23 ± 0.33
20	17.60 ± 1.45	3.69 ± 0.29	5.40 ± 0.00
28	29.60 ± 0.33	3.86 ± 0.33	5.60 ± 0.01
35	34.33 ± 5.67	3.90 ± 0.33	5.60 ± 0.33
42	35.00 ± 2.67	4.23 ± 0.67	6.30 ± 0.67
50	38.33 ± 4.41	4.30 ± 0.67	6.30 ± 0.09
58	42.66 ± 2.67	7.60 ± 0.33	11.33 ± 0.67
65	38.33 ± 1.20	4.30 ± 0.67	8.00 ± 1.15
80	35.00 ± 2.65	4.20 ± 0.33	8.00 ± 0.57
95	32.33 ± 2.33	3.00 ± 0.29	7.00 ± 0.33
110	31.33 ± 0.71	3.00 ± 0.58	7.00 ± 0.87
125	28.33 ± 4.41	2.00 ± 0.29	6.67 ± 0.88
140	26.67 ± 1.67	1.80 ± 0.12	6.00 ± 0.29
155	25.67 ± 1.45	1.67 ± 0.67	5.66 ± 0.67

Table 6. Correlation coefficient between pairs

Age and body weight	0.968**
Age and per cent weight of bursa (to body weight)	-0.841**
Weight of bursa and age	0.534*
Weight of bursa and body weight	0.684**
Weight of bursa and length of bursa	0.963**
Weight of bursa and diameter (top) of bursa	0.567*
Weight of bursa and diameter (bottom) of bursa	0.586*

* $P<0.05$ ** $P<0.01$

Table 7. Number and size of the lymphoid follicles, length, height and width of the plica, extent of cortex and medulla and lymphocyte population of lymphoid follicles of bursa of Fabricius in different age groups of White Pekin ducks (Mean ± S.E.)

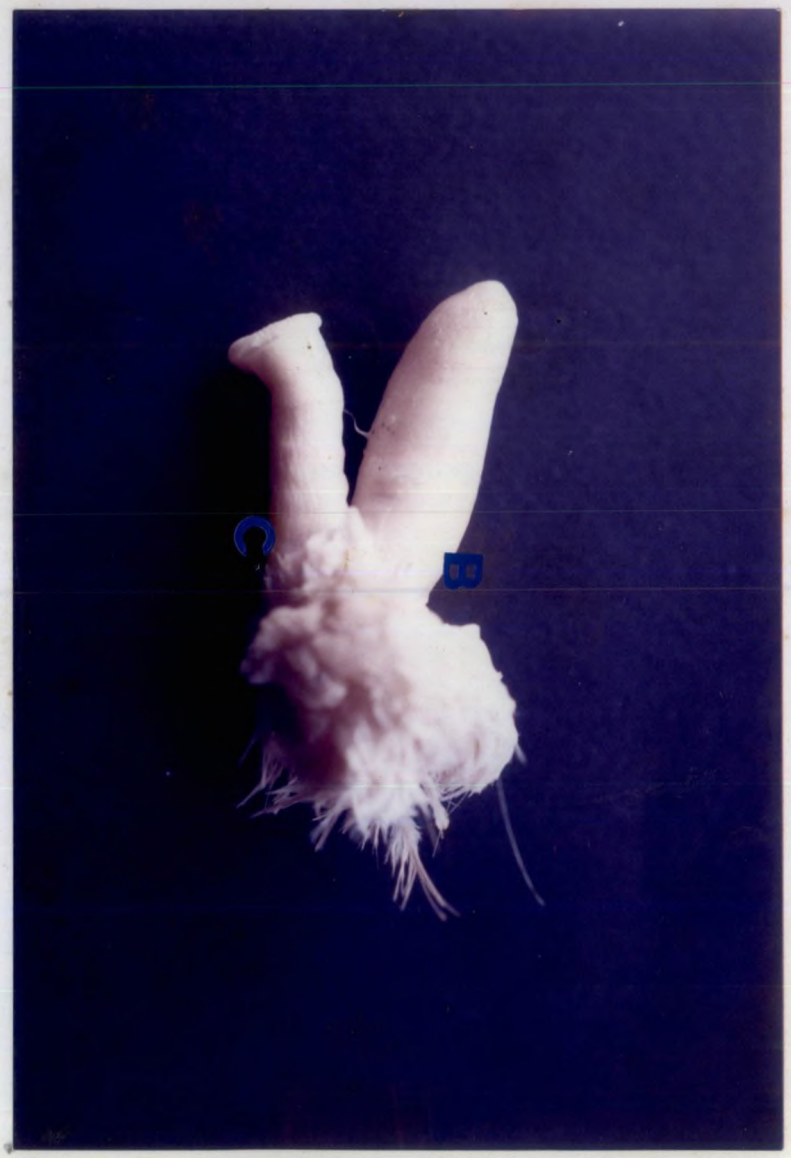
Age	Number of lymphoid follicles	Diameter of lymphoid follicles (μm)	Number of plicae	Length of plicae (μm)		Width of plicae (μm)		Width of cortex (μm)	Diameter of medulla	Number of lympho-cytes per follicle
				Large	Small	Large	Small			
0	150	162.75±1.86	6	1575.50±1.11	350.0±1.13	525.0±1.28	507.5±0.86	43.75±0.83	70±0.22	890
5	155	172.50±2.11	6	1607.50±2.32	350.01.86	587.5±1.38	567.5±0.92	52.91±0.86	111.5±0.83	1040
10	155	246.750±0.24	8	1802.50±2.43	375.03.21	700.0±1.11	660.0±0.63	53.75±0.12	163.30.28	1170
15	155	163.75±4.01	8	2012.50±2.32	525.0±3.63	770.0±1.62	765.0±0.66	54.84±1.28	170±0.36	1410
20	155	272.50±1.54	8	2875.00±1.86	525.0±3.28	787.5±0.86	770.0±0.62	53.37±1.32	174.5±0.08	1740
28	160	364.58±3.26	11	2975.00±1.32	700.0±3.11	840.0±0.93	790.0±0.55	87.5±1.26	241.5±0.92	2290
35	160	369.66±0.65	11	3325.00±1.43	1015.0±3.11	1050.0±0.66	1225.0±0.89	91.37±1.83	257.25±1.36	3300
42	160	523.25±0.32	11	3500.00±1.88	1400.0±3.36	1225.0±0.86	1262.5±1.28	98.75±1.22	382.1±1.82	3400
50	160	609.52±1.63	11	3800.00±1.86	1575.0±1.23	1250.0±1.99	1250.0±0.88	100.42±1.36	397.5±1.22	3700
58	160	621.25±1.87	11	4462.50±2.36	1600.0±1.11	1662.5±1.13	1365.0±1.38	110.58±1.28	408.33±1.83	4500
65	165	605.80±2.23	11	4300.00±0.08	1568.0±1.11	1514.5±0.33	1342.0±0.96	99.58±1.68	396.66±1.26	4300
80	158	595.80±2.56	11	4375.00±0.31	1512.5±1.32	1468.5±0.68	1315.0±0.12	86.16±2.21	375.42±1.92	4250
95	150	580.75±1.87	8	4200.00±0.28	1225.0±1.36	1400.0±0.89	1315.0±1.11	79.58±0.08	355.83±1.28	3990
110	150	560.00±0.65	8	4198.00±0.98	1200.0±1.16	1387.5±0.89	1300.0±1.28	64.16±0.26	342.6±1.98	3620
125	140	533.75±1.54	8	4000.001.18	1215.0±2.21	1213.3±0.32	1287.51±1.11	52.5±0.83	336.2±1.22	3110
140	120	430.22±0.24	8	3800.251.12	1128.0±2.31	1108.5±0.44	005.0±0.33	38.75±0.22	318.250.92	2740
155	60	180.12±1.66	6	3200.25±1.32	940.0±2.63	980.5±0.53	821.0±0.33	16.05±0.92	159.5±0.68	860

Fig.1. The bursa of Fabricius in duck (28 days)

Left : External appearance
Right : Internal surface, showing plicae

Fig.2. The bursa of Fabricius in duck showing its relation with cloaca (28 days)

C - Cloaca
B - Bursa of Fabricius


C
B

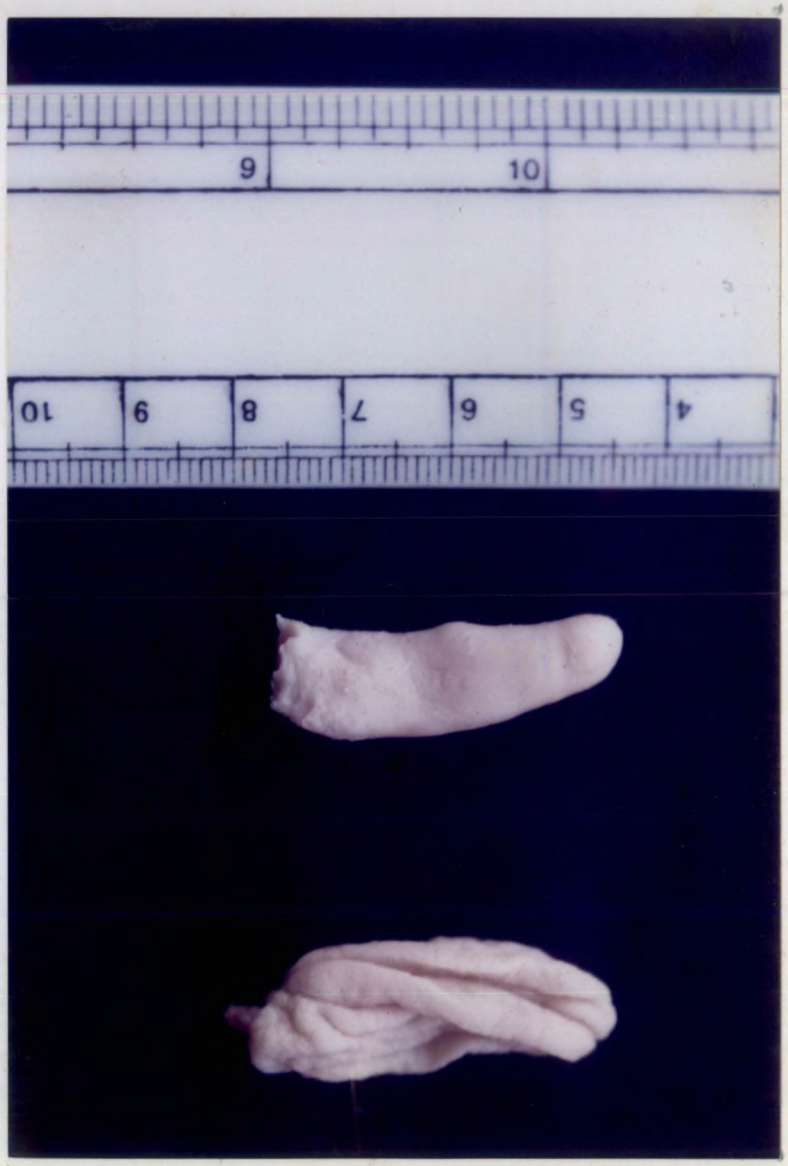

6
10
10
9
8
7
6
5
4

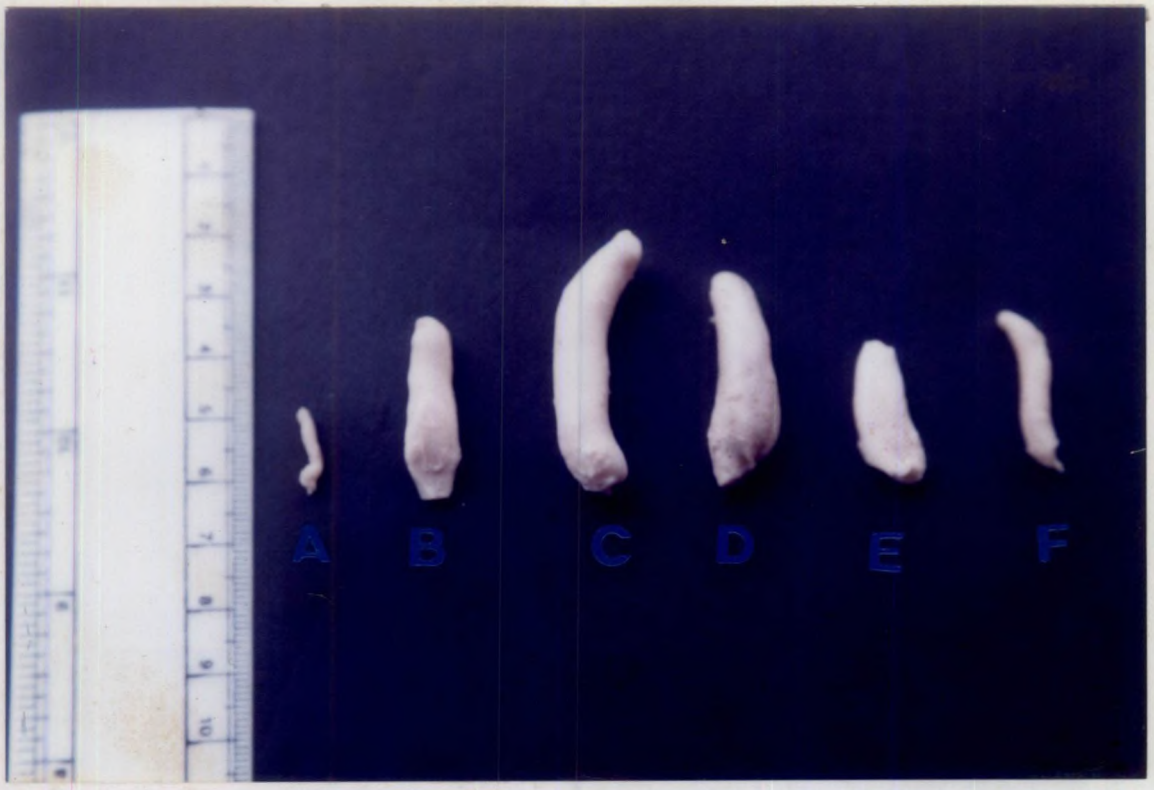
Fig.3. The bursa of Fabricius in White Pekin ducks at different ages

A : Day-old
B : 28 days-old
C : 58 days-old
D : 95 days-old
E : 125 days-old
F : 155 days-old

Fig.10. Section of bursa showing tunica mucosa with a lining epithelium and lamina propria filled with lymphoid follicles (35 days)

C : Cortex
M : Medulla
E : Epithelial zone

H&E x 160


A
B
C
D
E
F

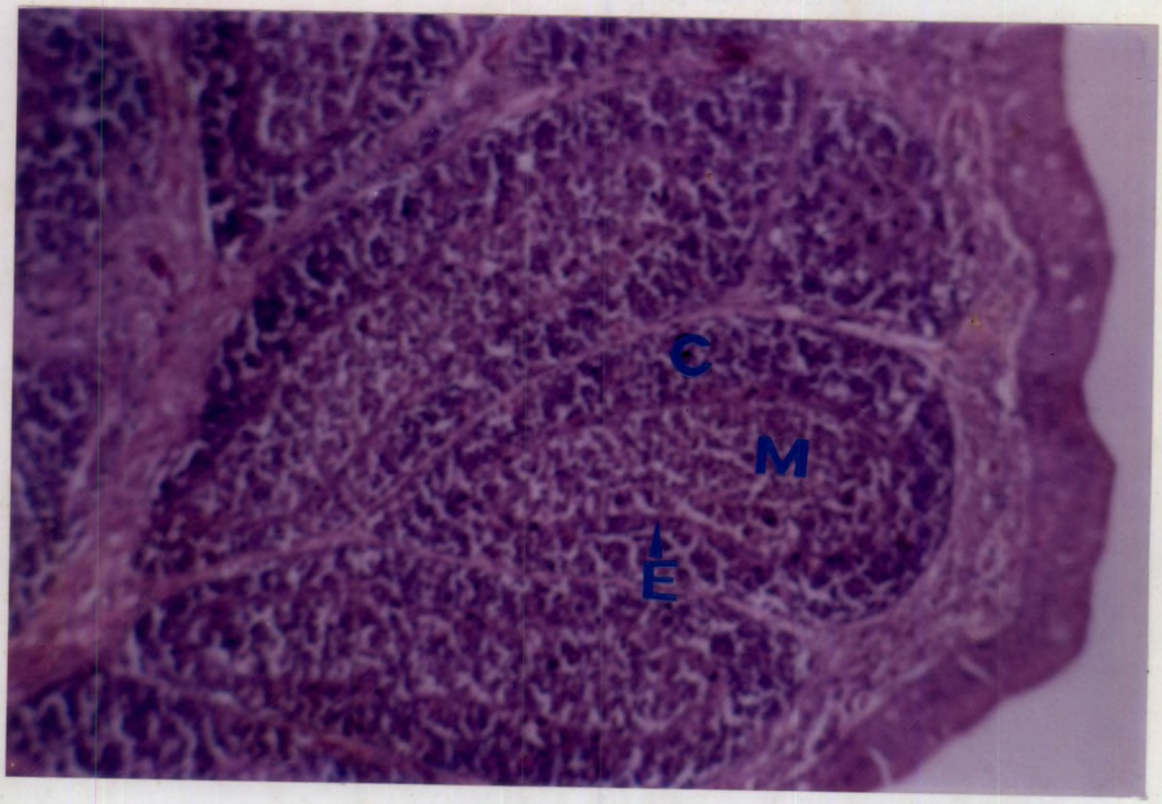

C
M
E

FIG. 4 THE GROWTH OF BURSA OF FABRICIUS IN WHITE PEKIN DUCKS

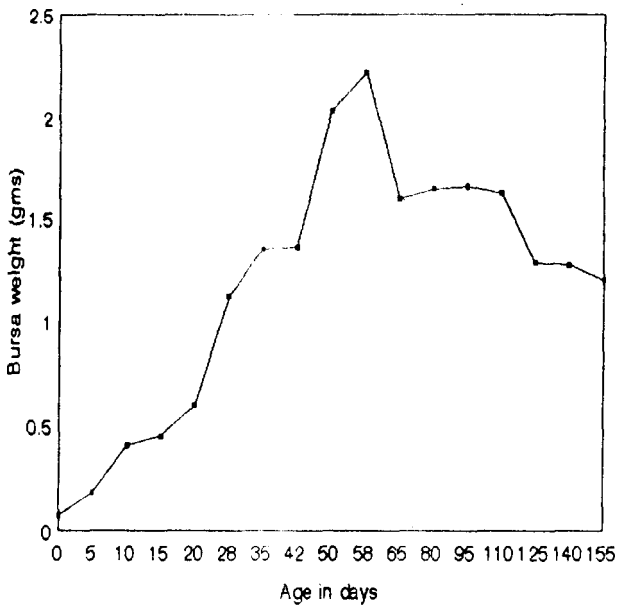


FIG.5 RELATIONSHIP BETWEEN AGE AND BODY WEIGHT

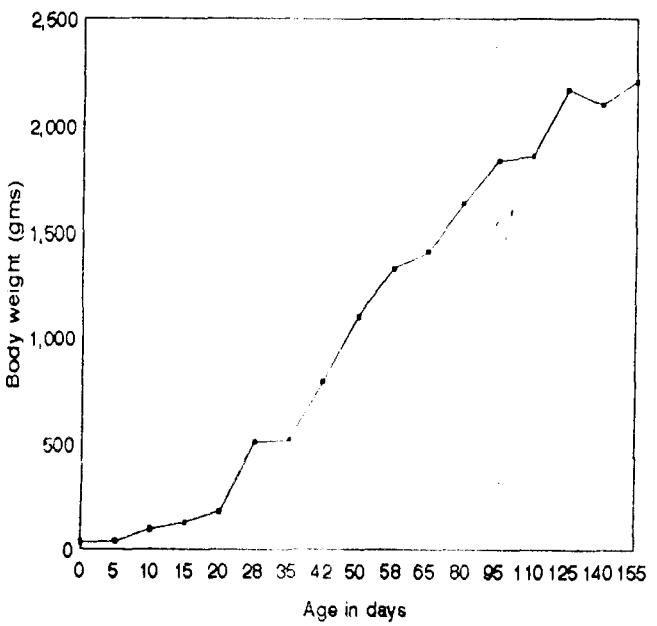


FIG 6 THE GROWTH OF BURSA OF FABRICIUS IN WHITE PEKIN DUCKS

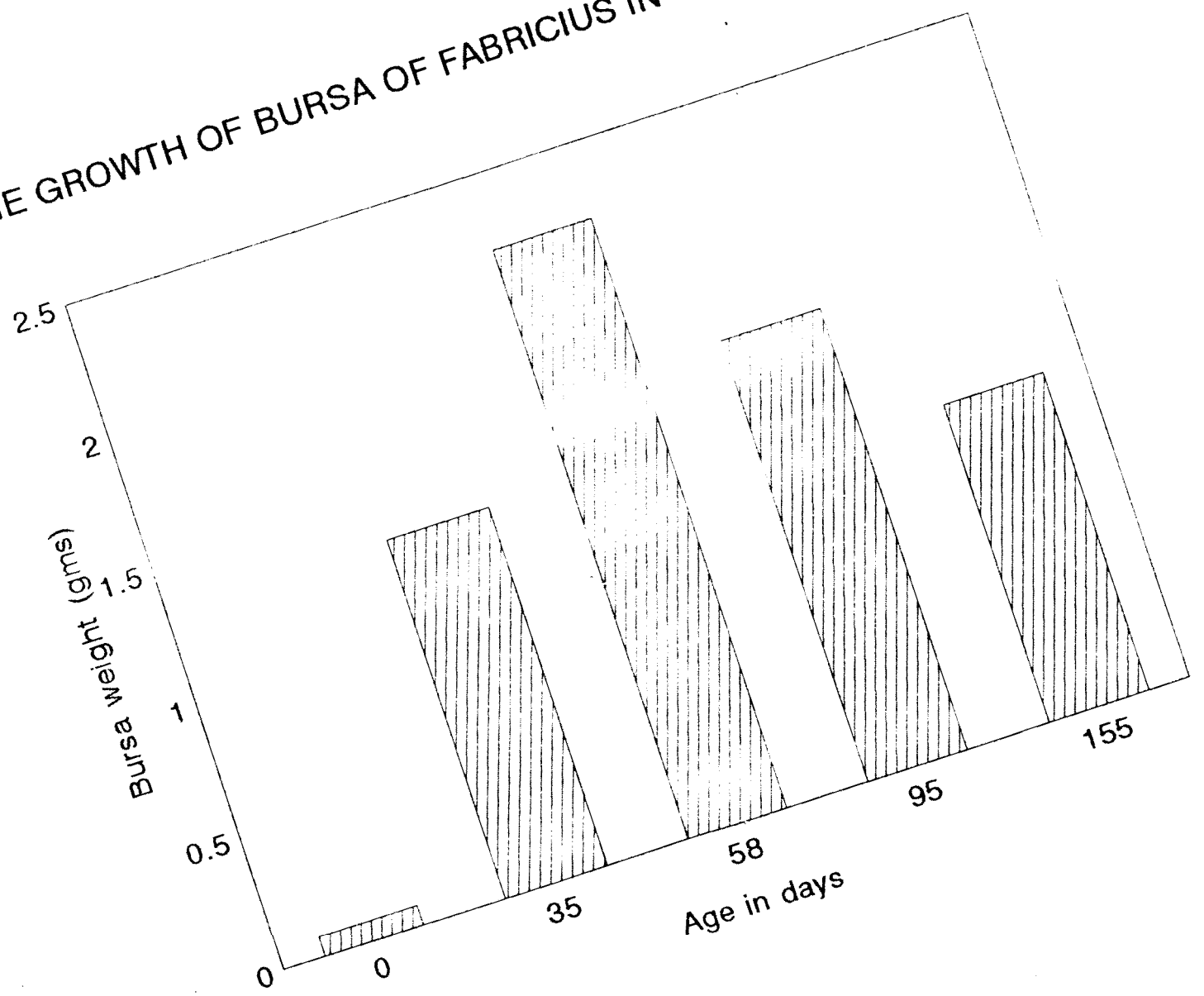


FIG. 7 BODY - BURSA WEIGHT RELATIONSHIP OF GROWING WHITE PEKIN DUCKS

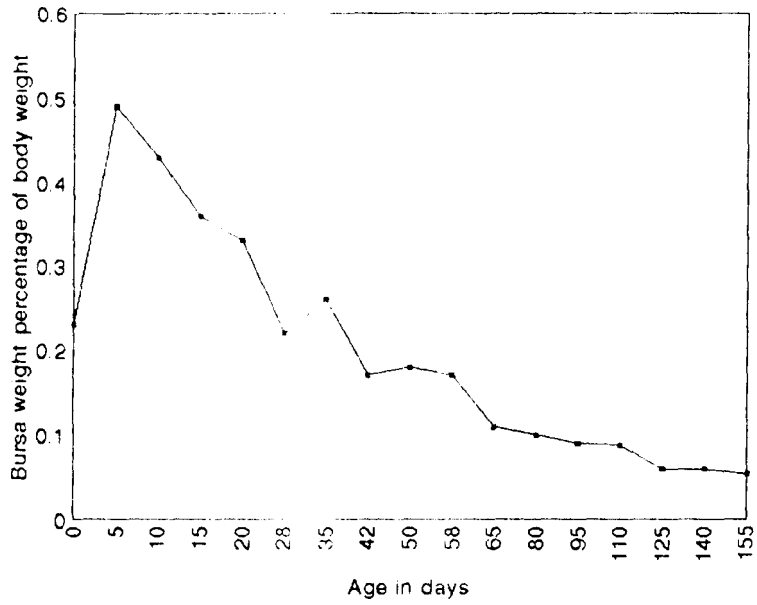


FIG. 8 RELATIONSHIP BETWEEN BODY WEIGHT AND BURSA WEIGHT

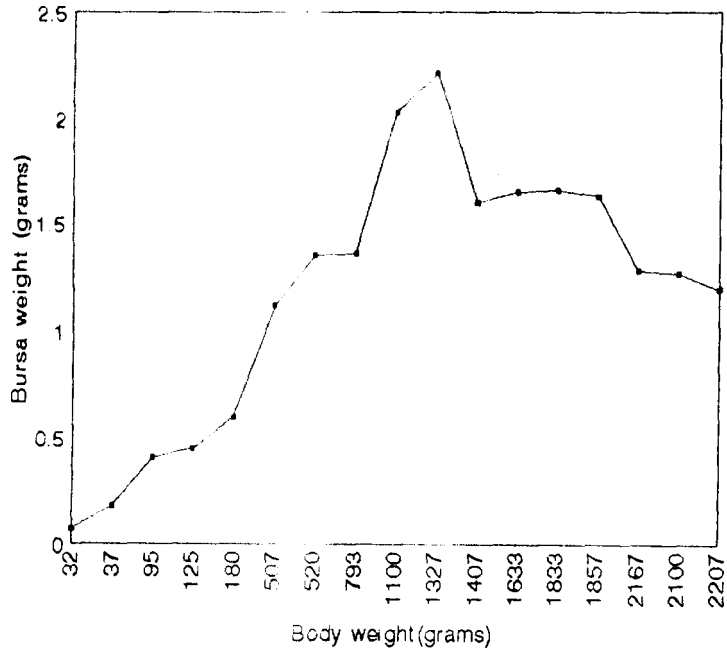


FIG.9 RELATIONSHIP BETWEEN AGE AND SIZE OF BURSA OF FABRICIUS

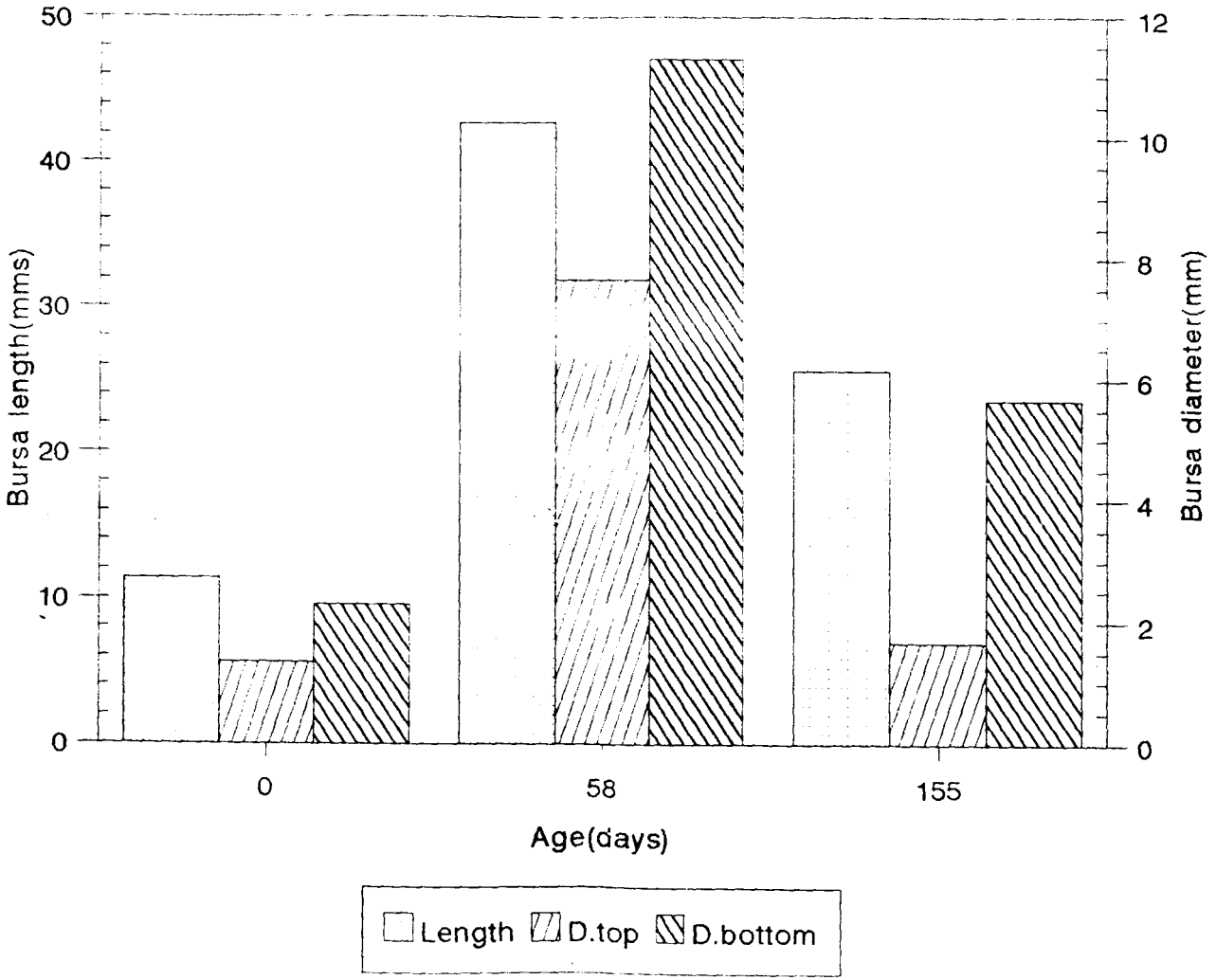


Fig.11. Section of bursa showing lining epithelial cells of bursal mucosa (35 days)

H&E x 160

Fig.12. Section of the capsule of bursa showing outermost serosa, muscularis composed of inner longitudinal and outer circular muscle layers. In the inner connective tissue is found large blood vessels (58 days)

Mallory's PTAH Staining x 160

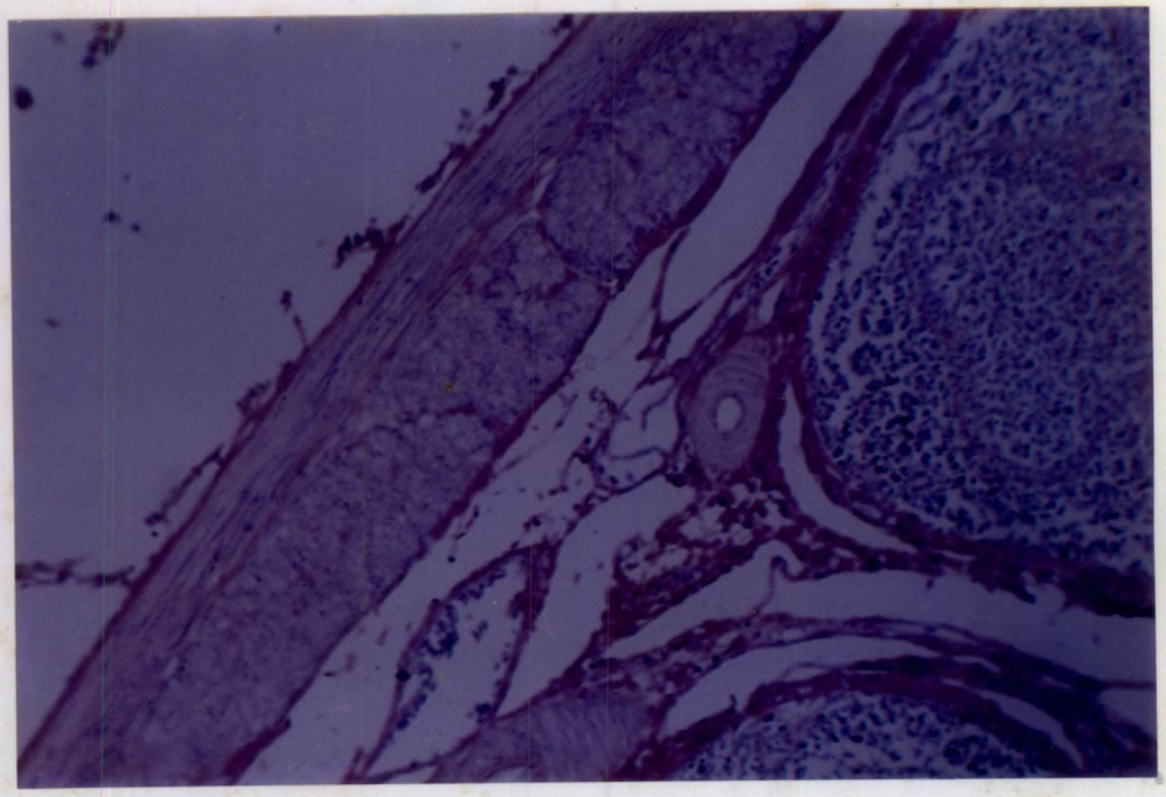
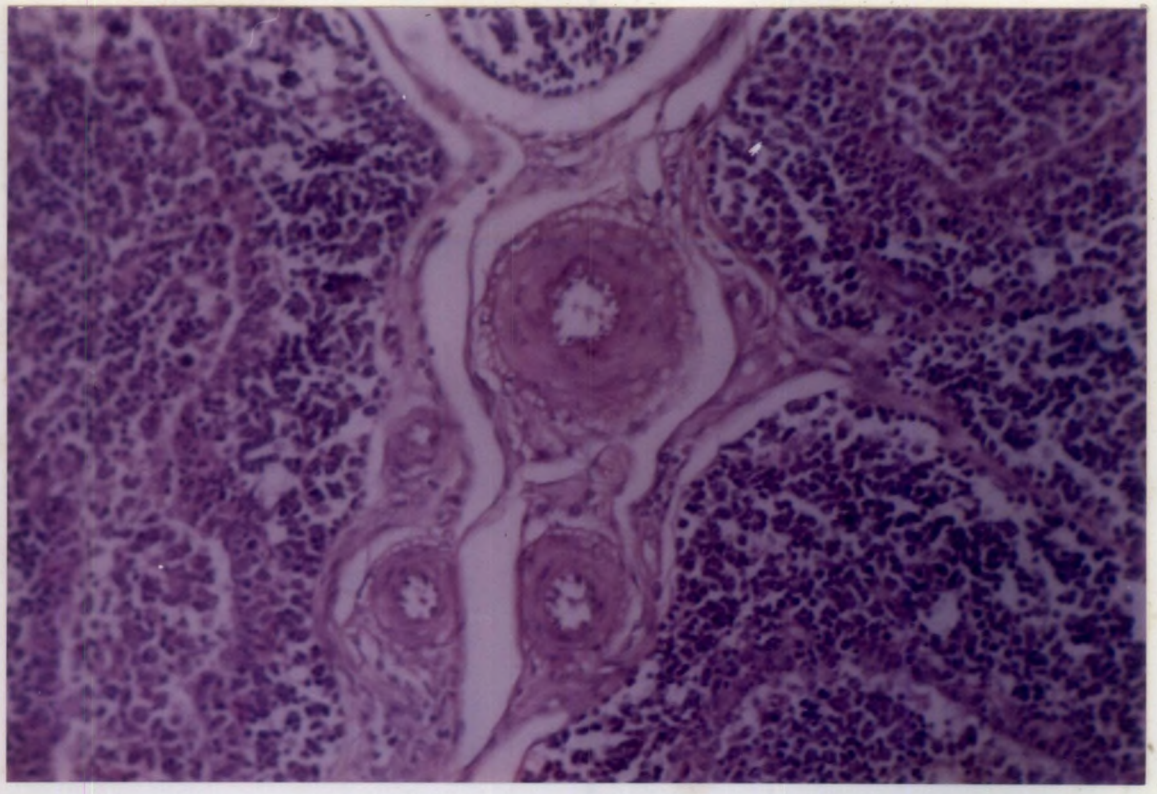


Fig.13. Section of bursa showing large blood vessels in the interfollicular connective tissue (65 days)

H&E x 160

Fig.14. Section of bursa showing well-developed capillary network (N) separating the cortex (C) and medulla (M) of a lymphoid follicle. Immediately subjacent to the capillary is a layer of undifferentiated epithelial cells (arrows) (58 days)

H&E x 1000



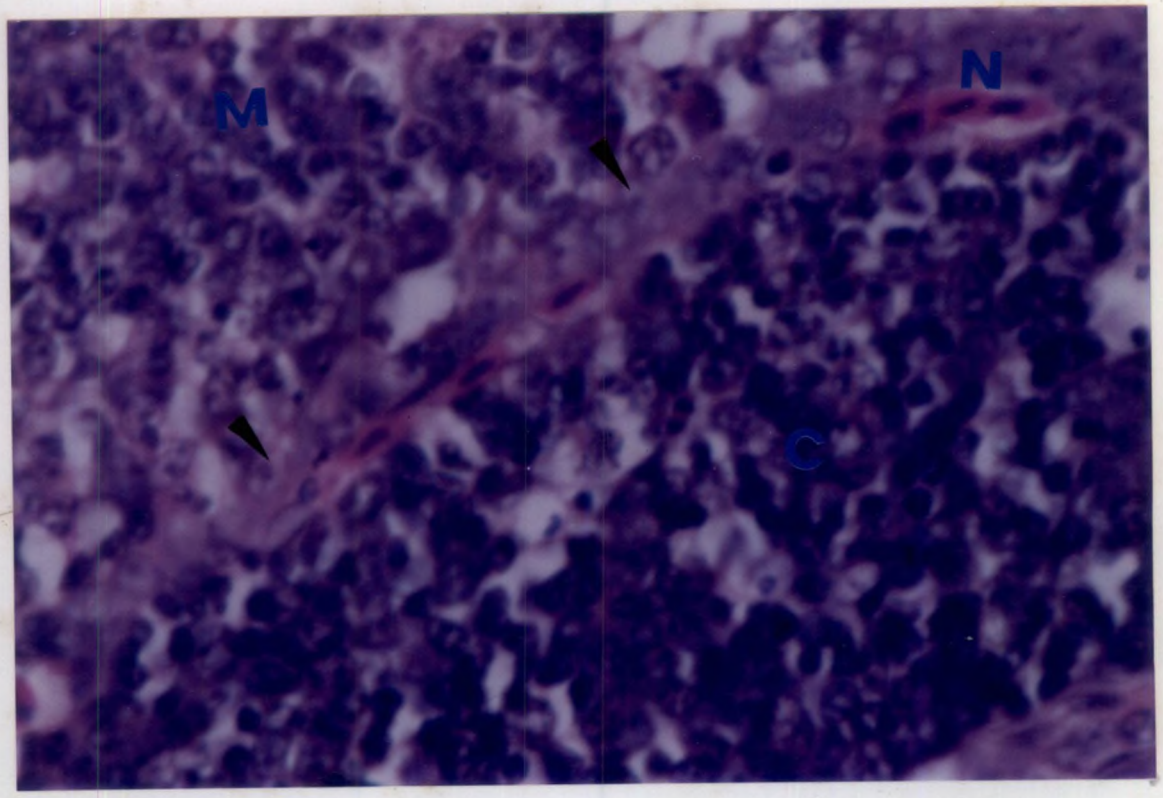
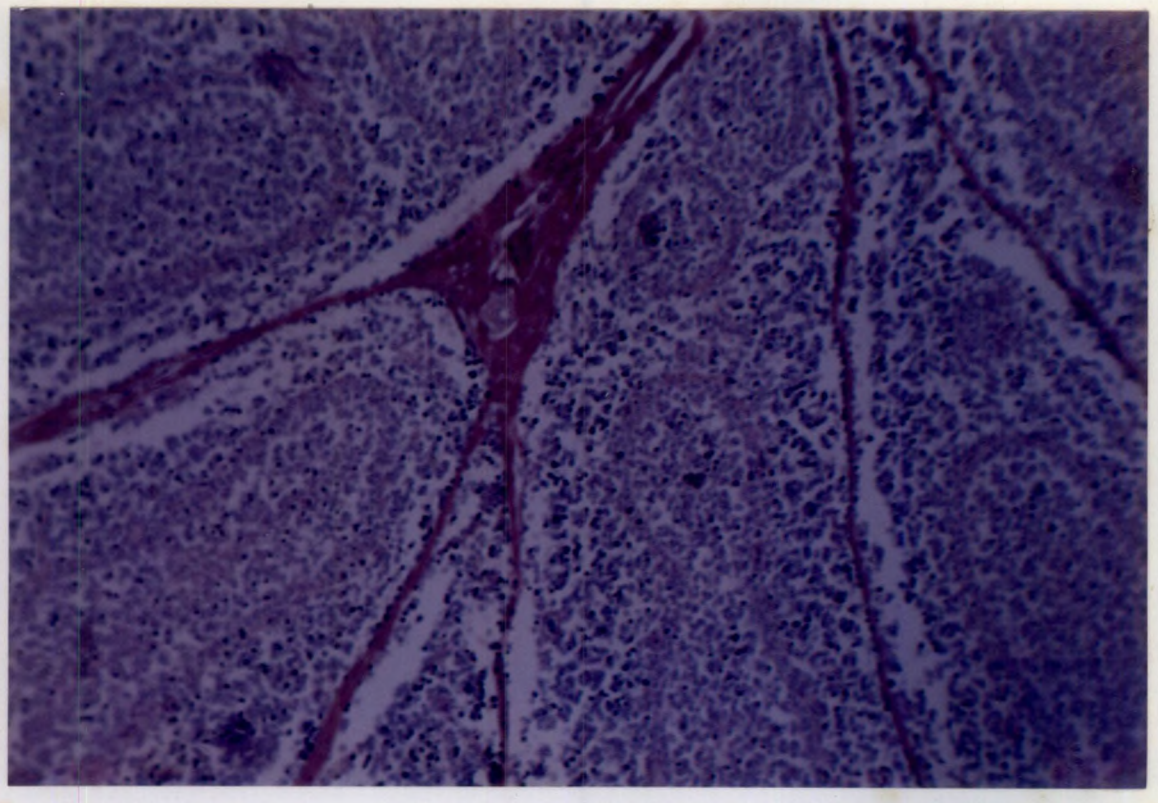

M
N
C

Fig.15. Section of bursa showing lymphoid follicles separated by connective tissue framework (125 days)

Mallory's PTAH staining x 160

Fig.16. Longitudinal section of bursa showing cloaco-bursal junction (58 days)

Mallory's PTAH Staining x 160



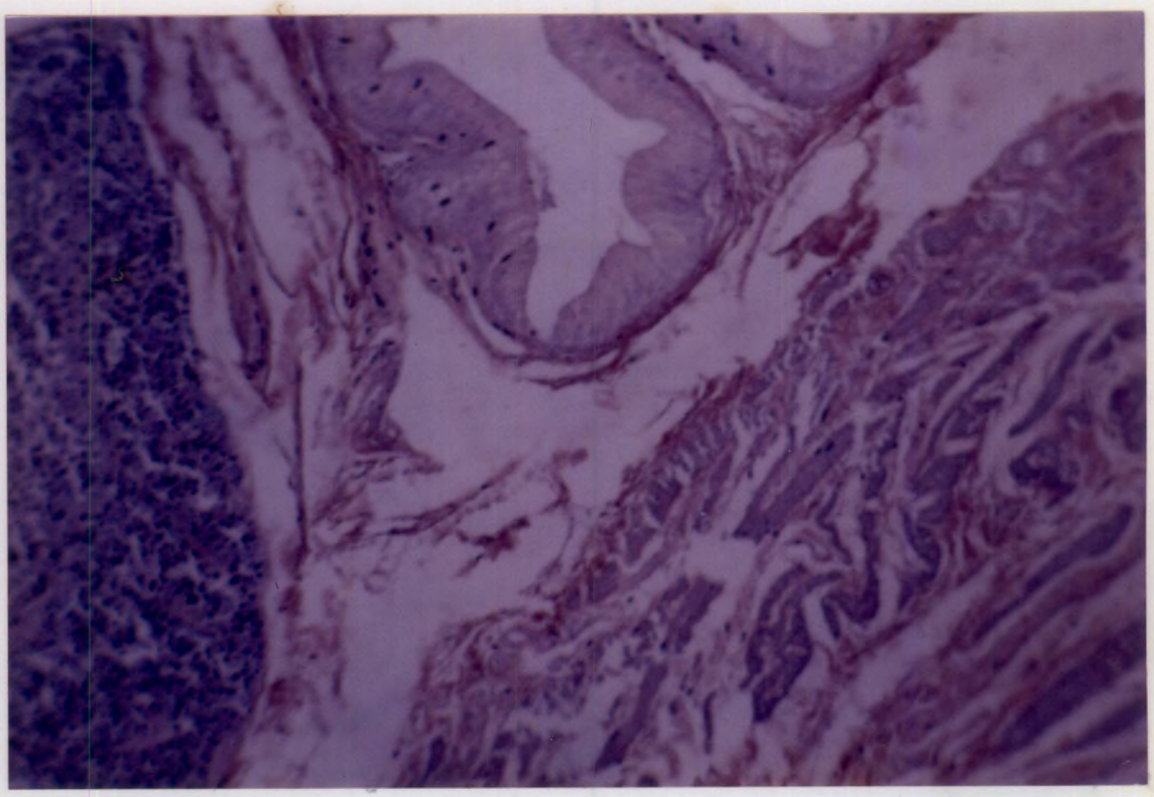
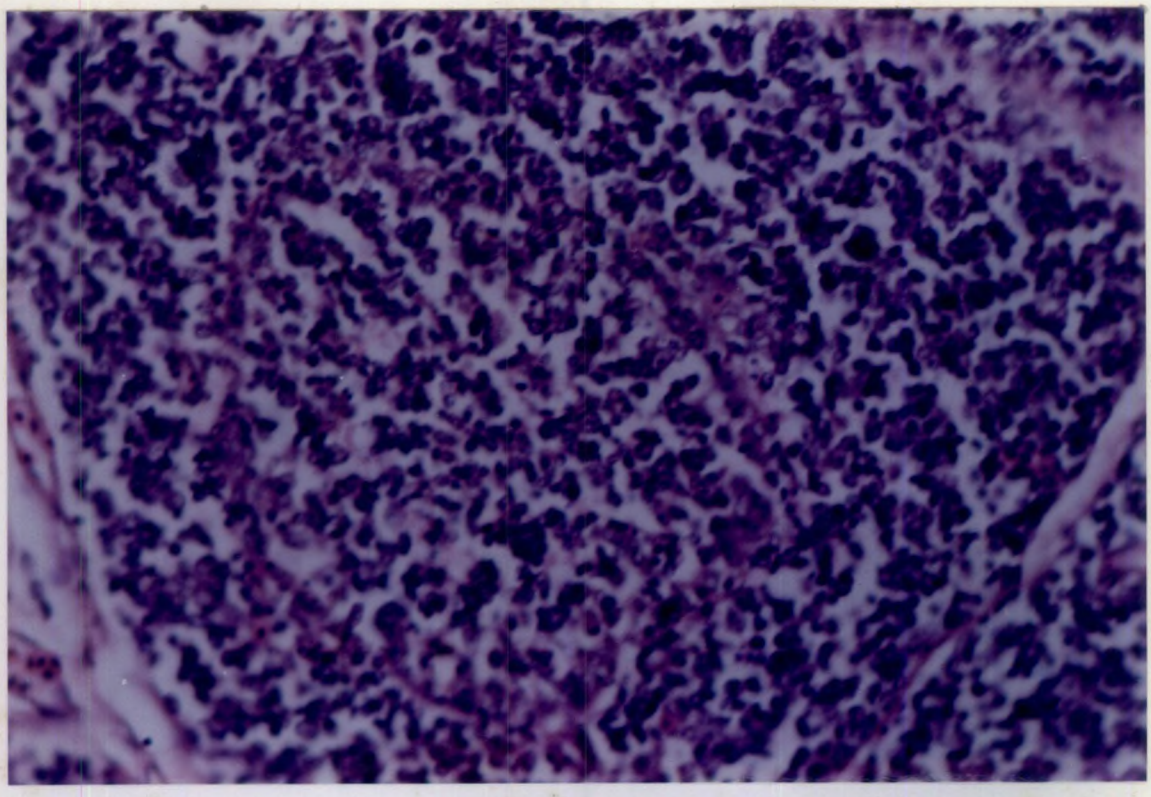


Fig.17. Section of bursa showing lymphocyte population in a follicle (day-old)

H&E x 250

Fig.18. Section of bursa showing lymphocyte population in a follicle (28 days)

H&E x 250



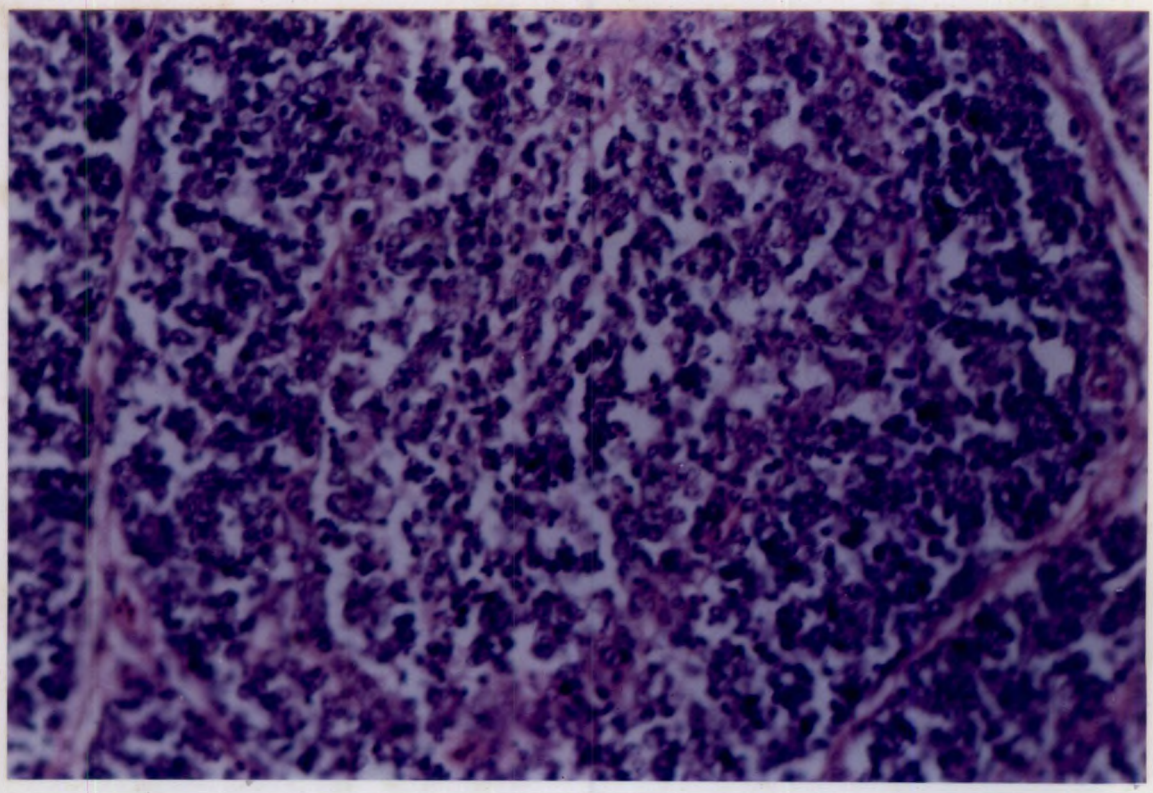
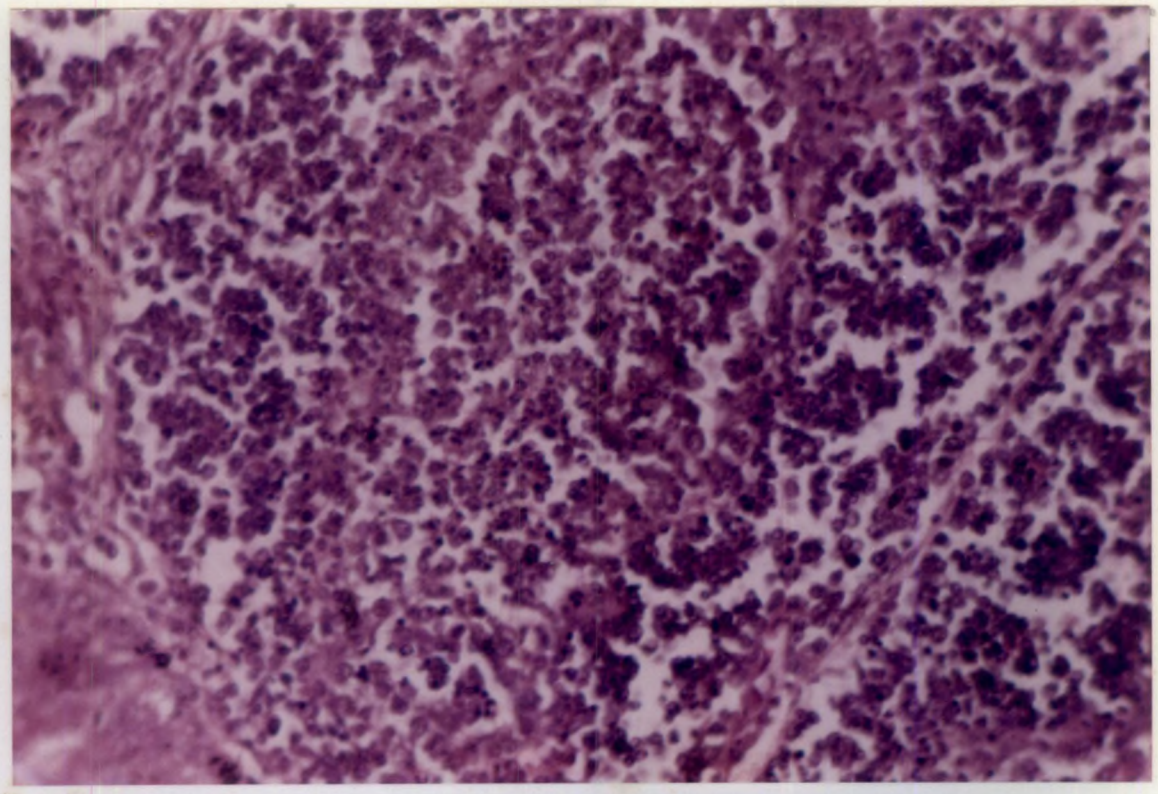


Fig.19. Section of bursa showing lymphocyte population in a follicle (58 days)

H&E x 250

Fig.20. Section of bursa showing lymphocyte population in a follicle (95 days)

H&E x 250



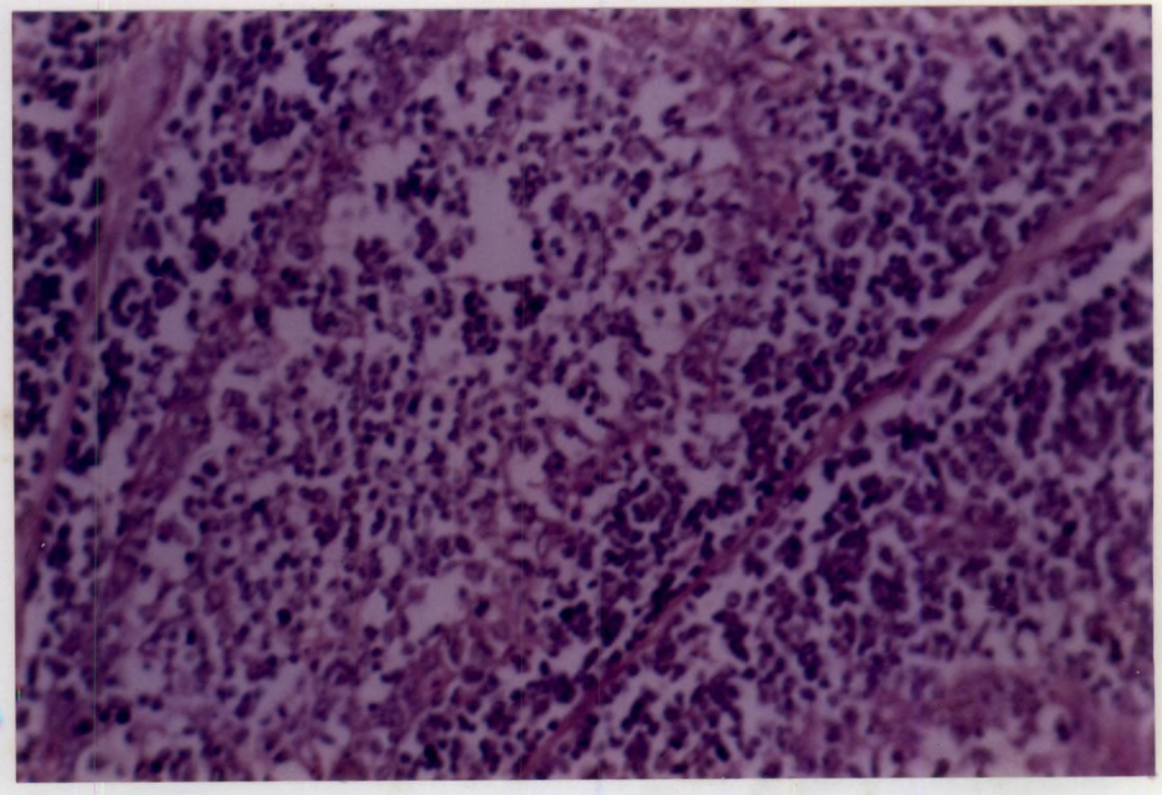
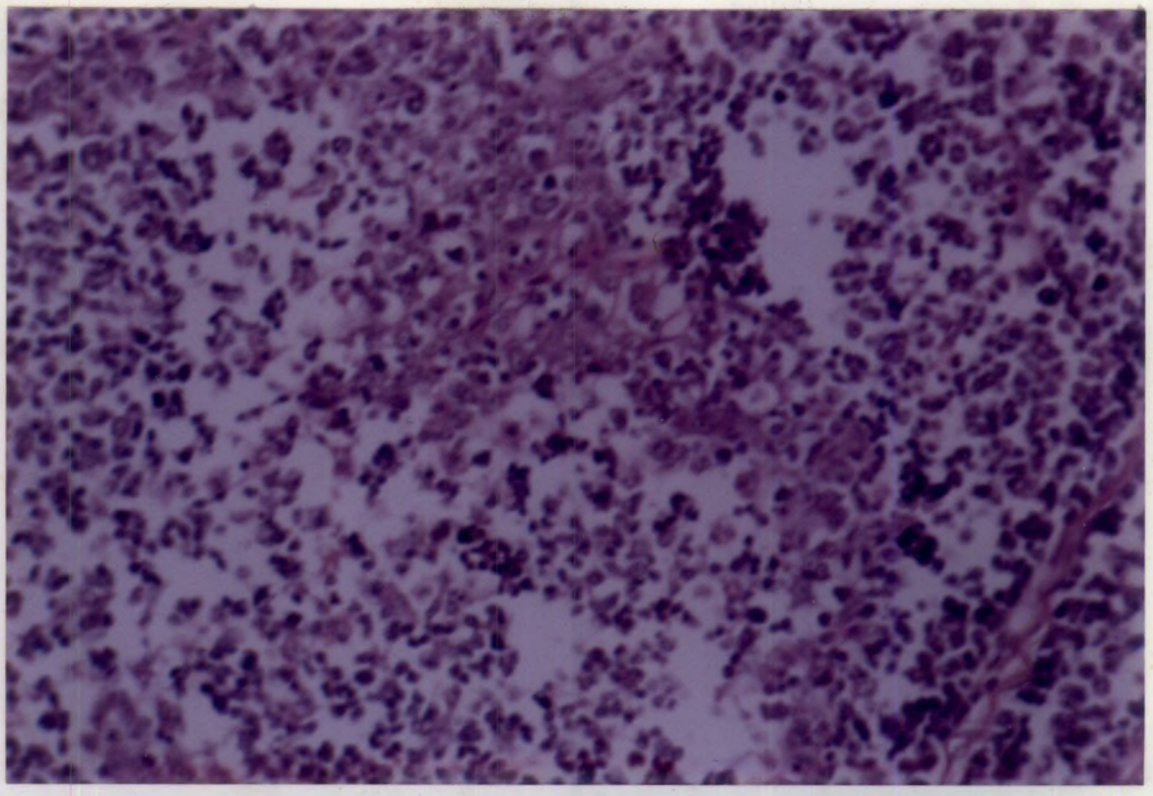


Fig.21. Section of bursa showing lymphocyte population in a follicle (125 days)

H&E x 250

Fig.22. Section of bursa showing lymphocyte population in a follicle (155 days)

H&E x 250



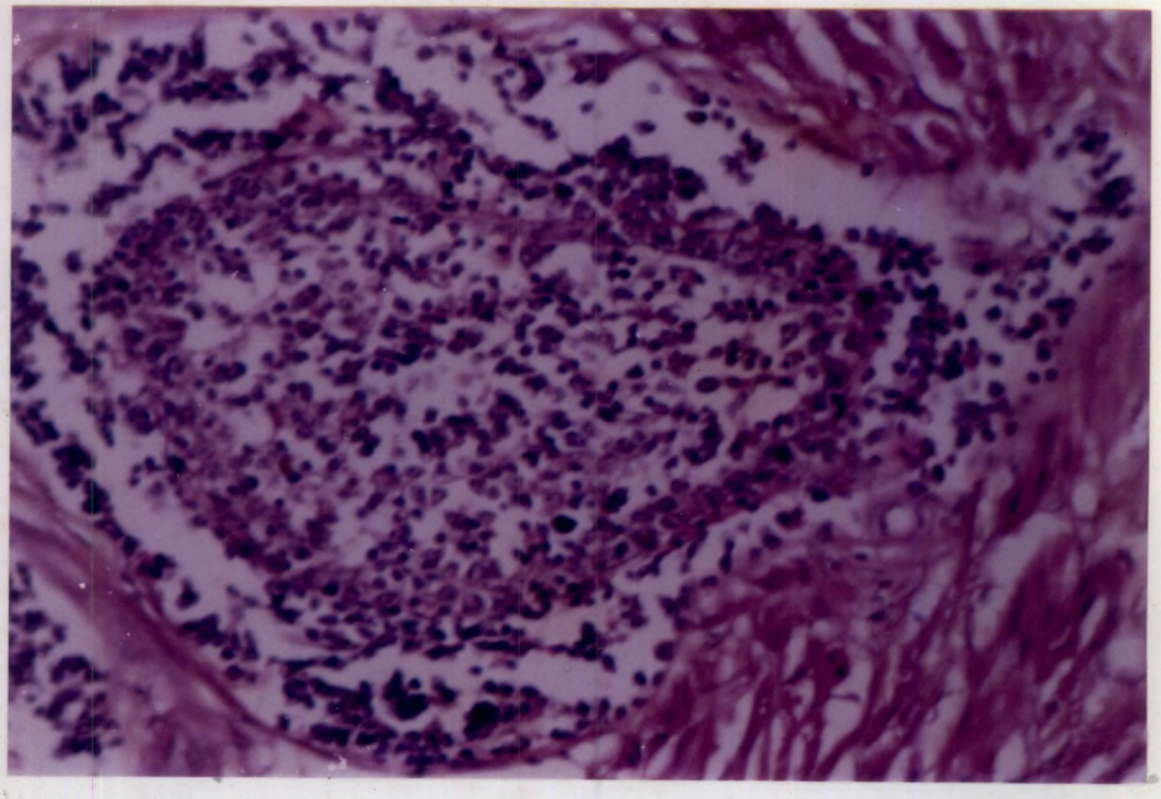


FIG.23 EFFECT OF AGE ON THE LYMPHOID FOLLICLES IN BURSA OF FABRICIUS

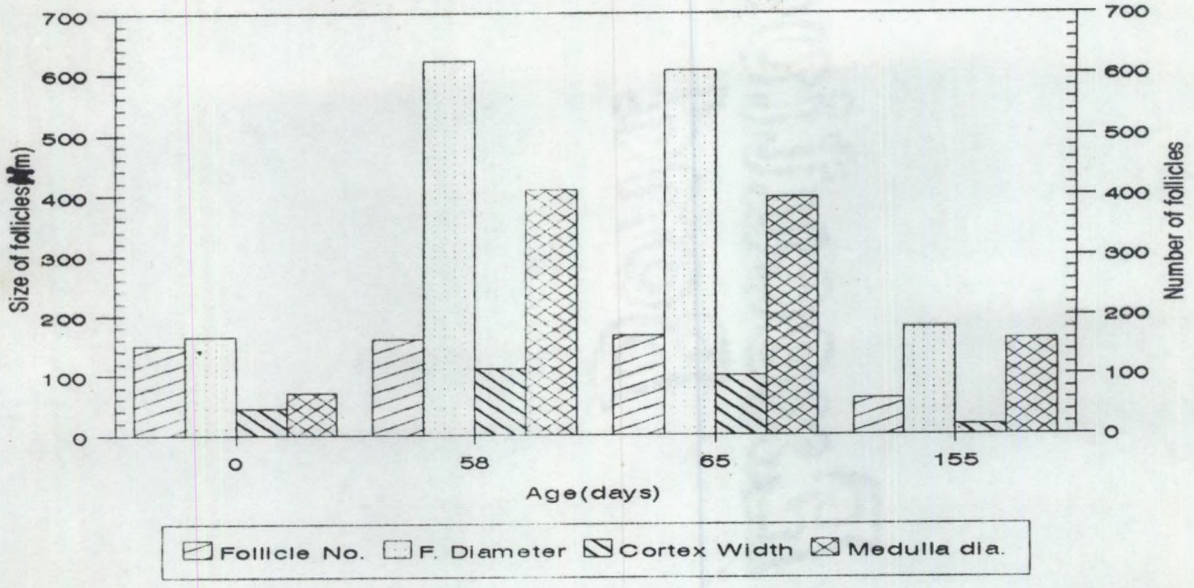


FIG.24 EFFECT OF AGE ON LYMPHOCYTE POPULATION IN FOLLICLES OF THE BURSA OF FABRICIUS

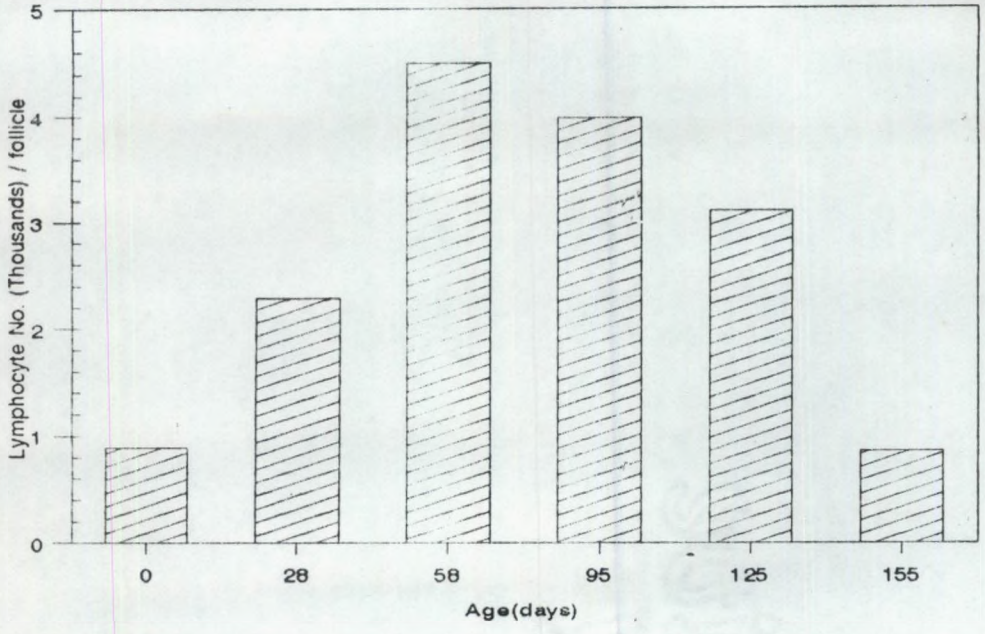
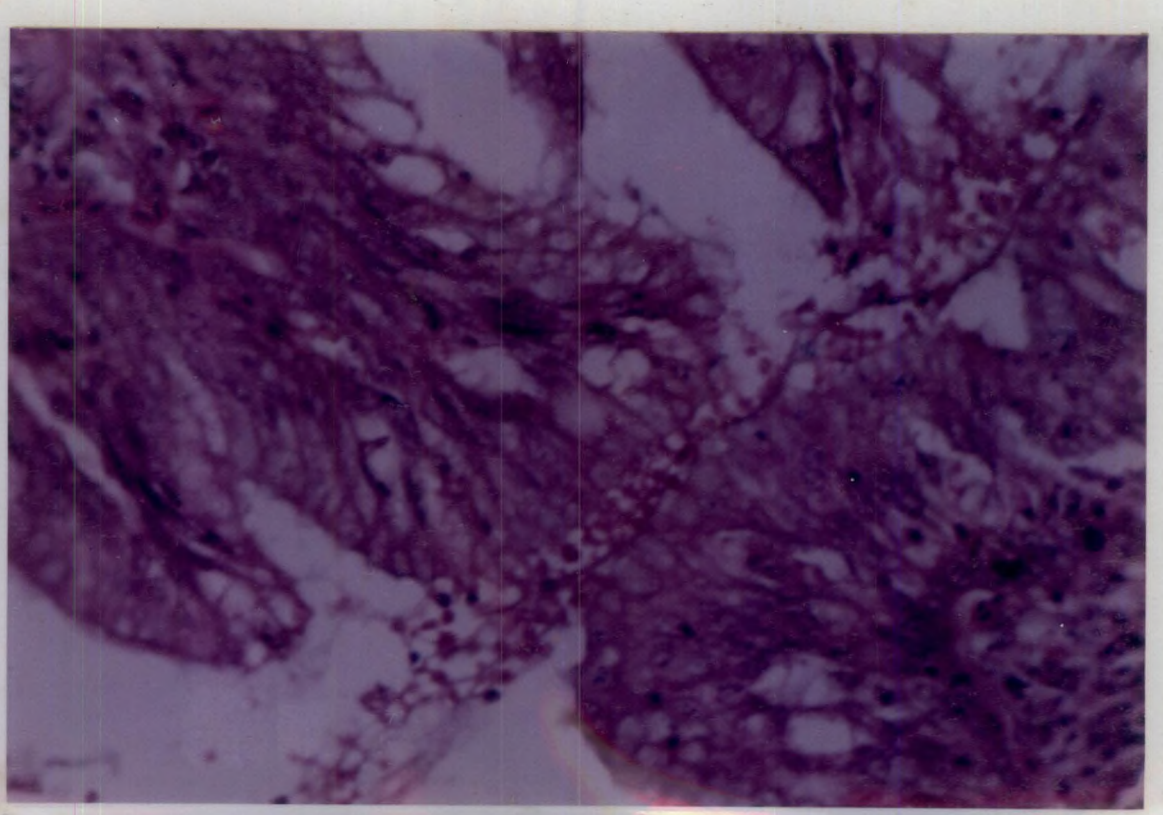


Fig.25. Section of bursa showing fusion of plicae (140 days)

H&E x 400

Fig.26. Section of bursa showing liquefactive necrosis of medullary and cortical tissue of the follicle (140 days)

H&E x 160



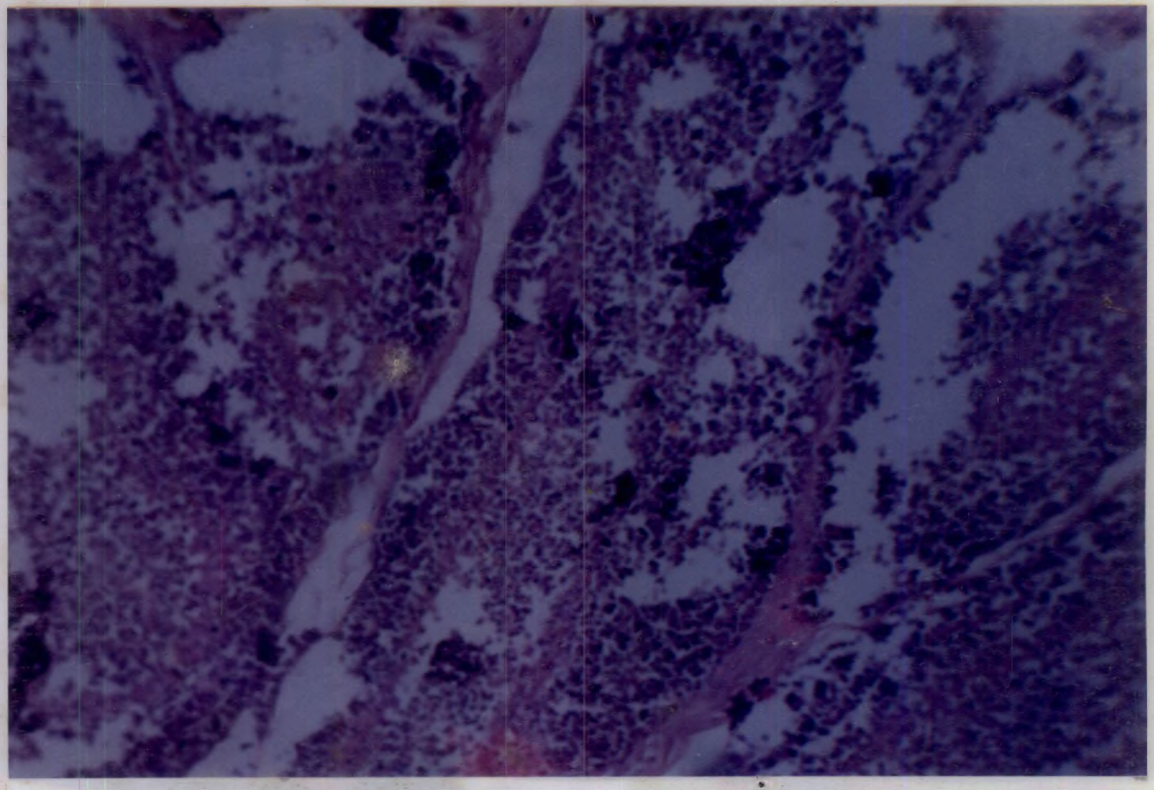
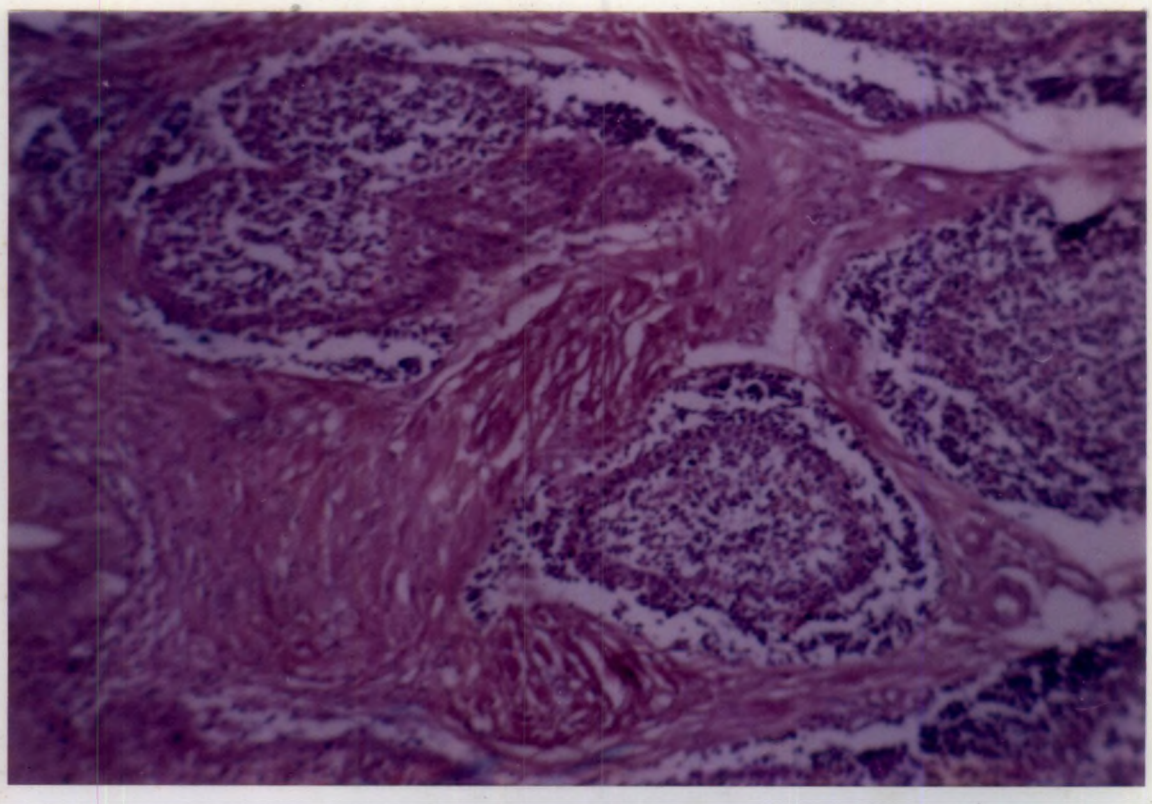


Fig.27. Section of bursa showing follicular atrophy and connective tissue proliferation (155 days)

H&E x 160

Fig.28. Section of bursa showing numerous coalescing degenerating follicles and fibrous connective tissue proliferation (155 days)

Mallory's PTAH Staining x 250



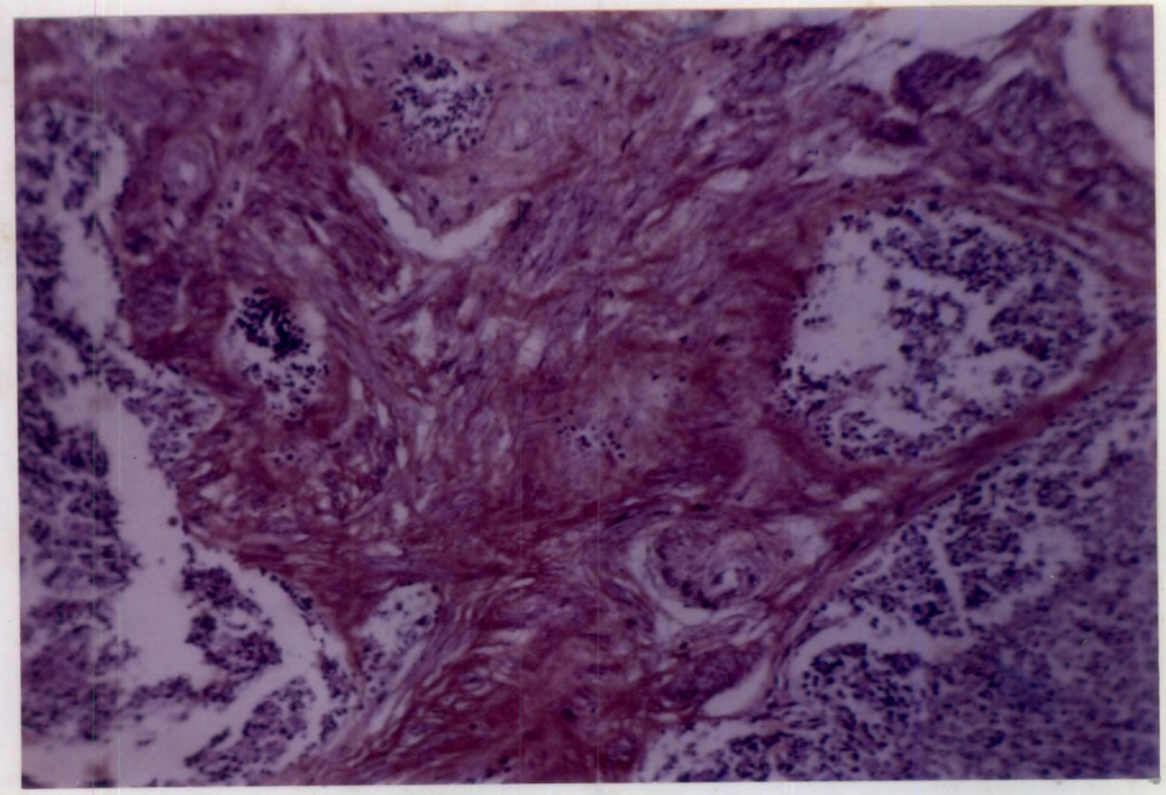
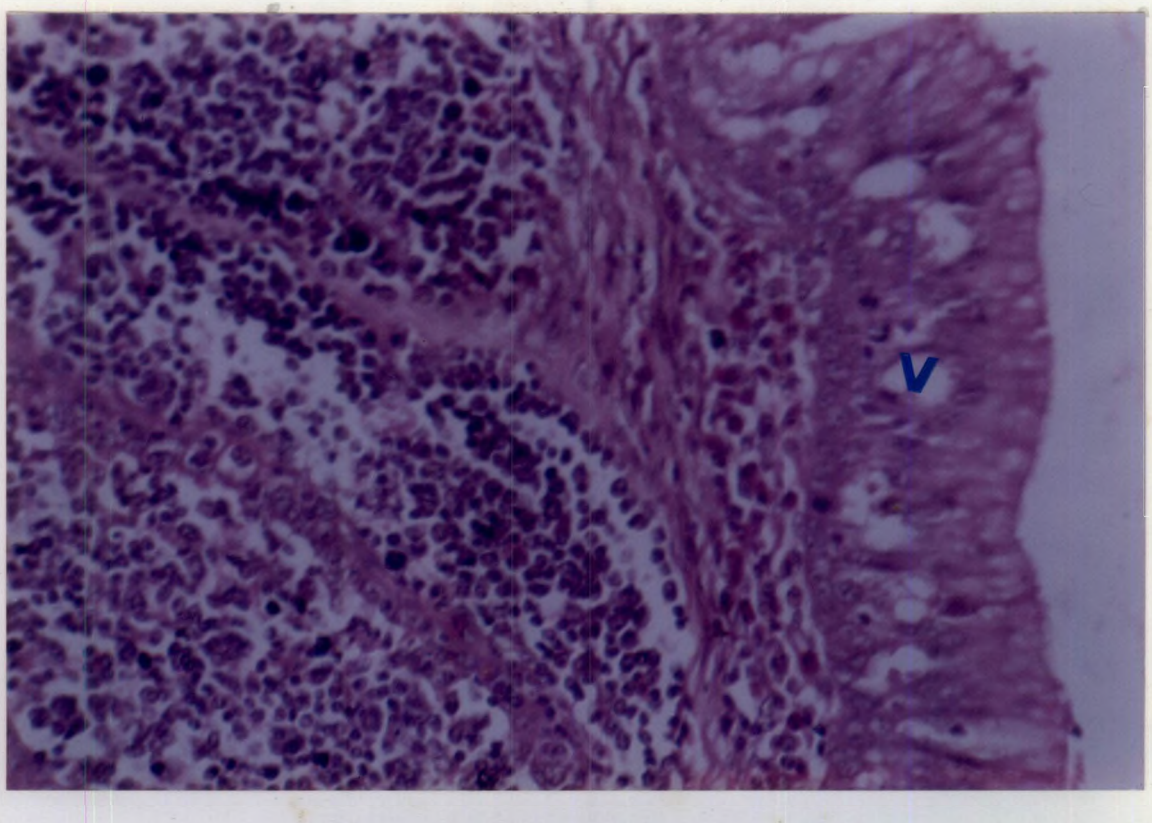


Fig.29. Section of bursa showing vacuolation (V)of epithelial cells (110 days)

H&E x 250

Fig.30. Section of bursa showing fusion of plical epithelium (140 days)

H&E x 160


V

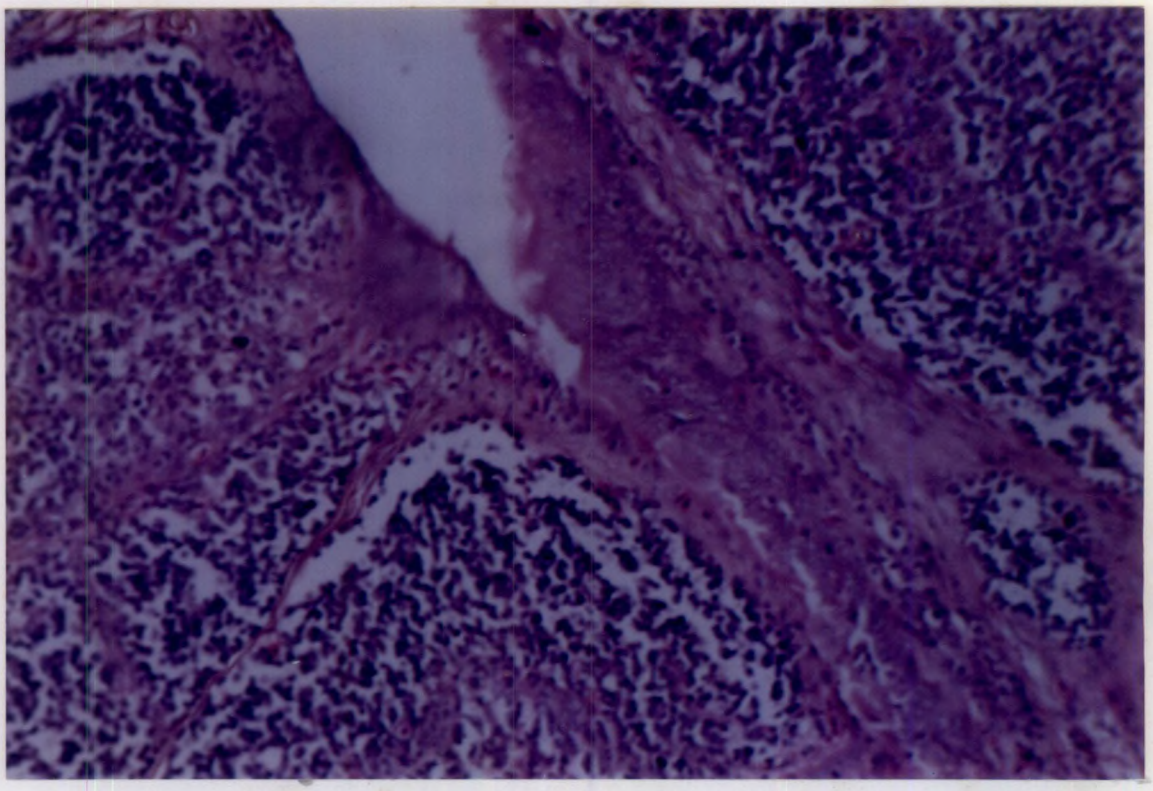
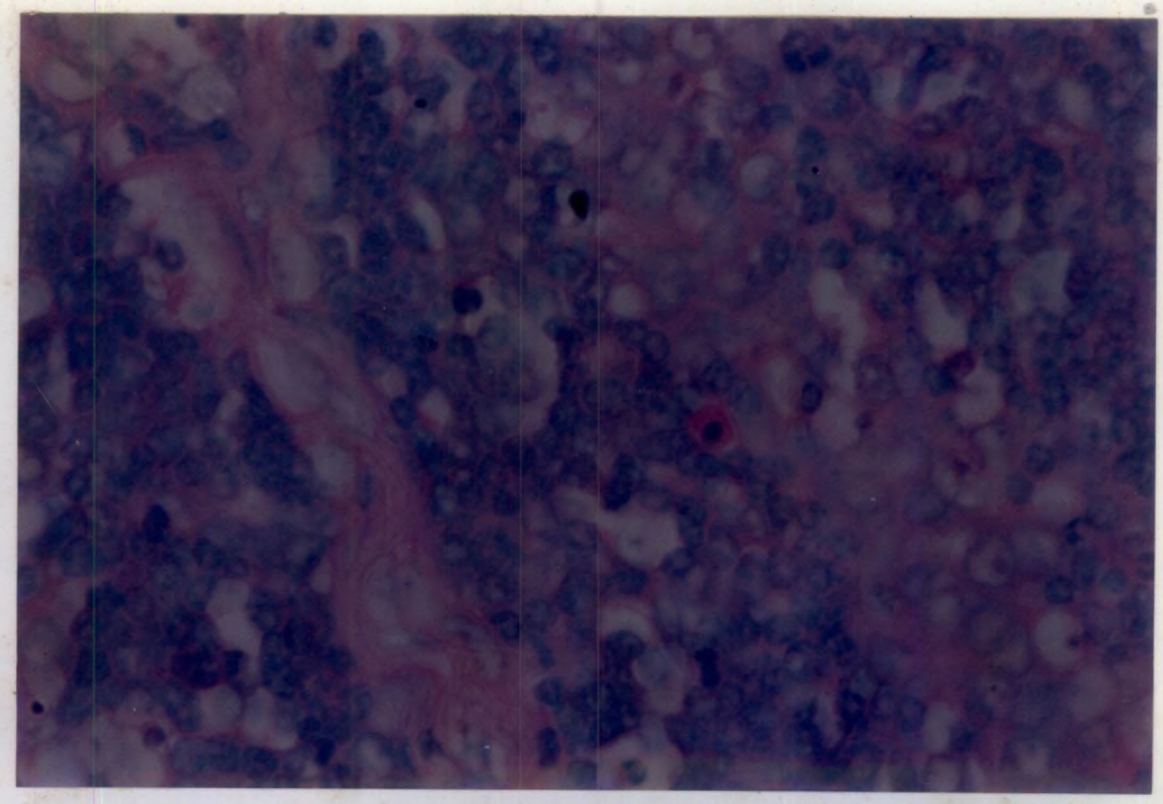


Fig.31. Section of bursa showing plasma cell in cortex of lymphoid follicles (20 days)

Methyl-Green Pyronin method x 1000

Fig.32. Section of bursa showing reticular fibres surrounding lymphoid follicles and in the corticomedullary junction (65 days)

Gridley's method x 100



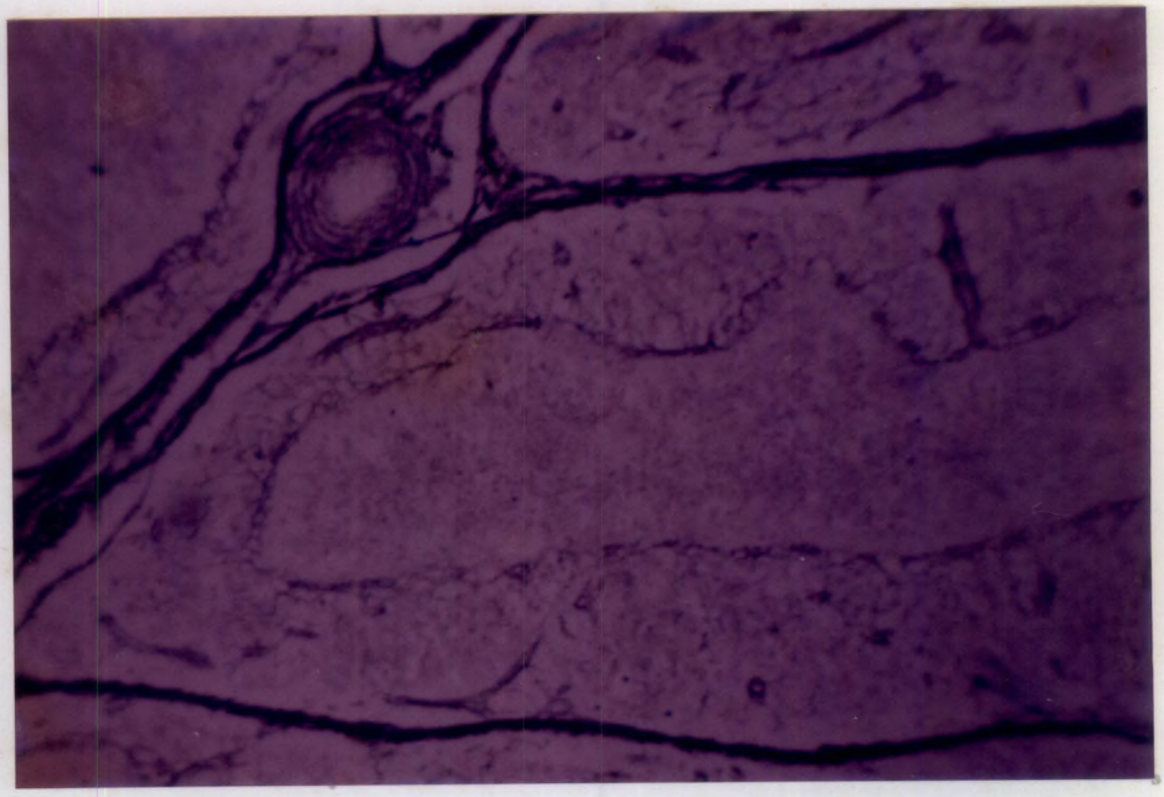
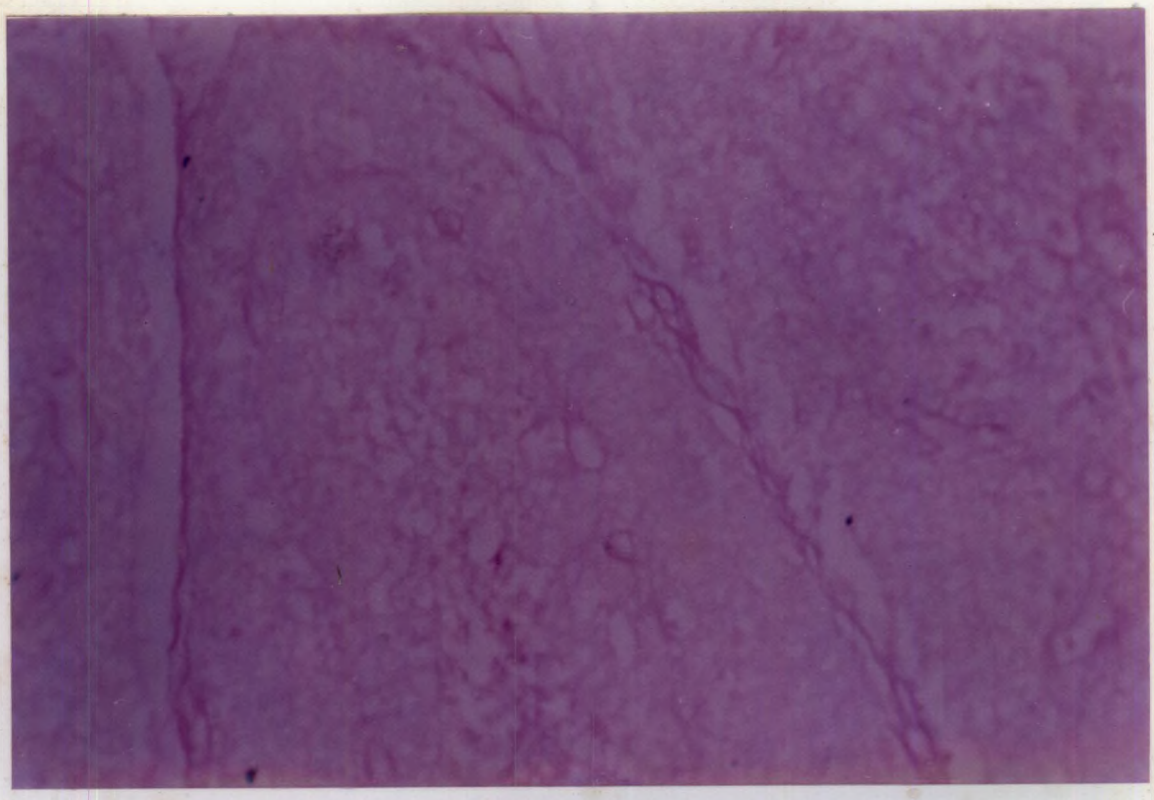


Fig.33. Section of bursa showing elastic fibres in the interfollicular connective tissue (58 days)

Gomori's Aldehyde Fuchsin method x 250

Fig.34. Section of bursa showing PAS positive lining epithelium with goblet cells (58 days)

PAS method x 250



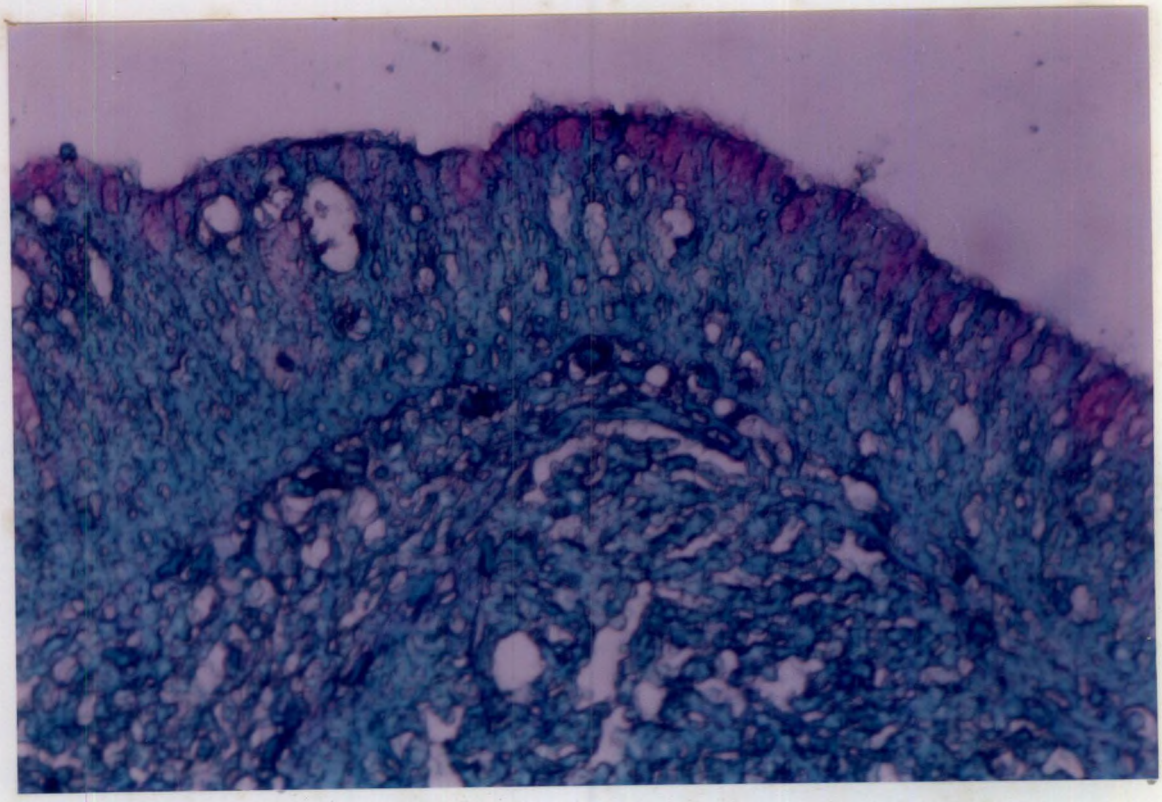
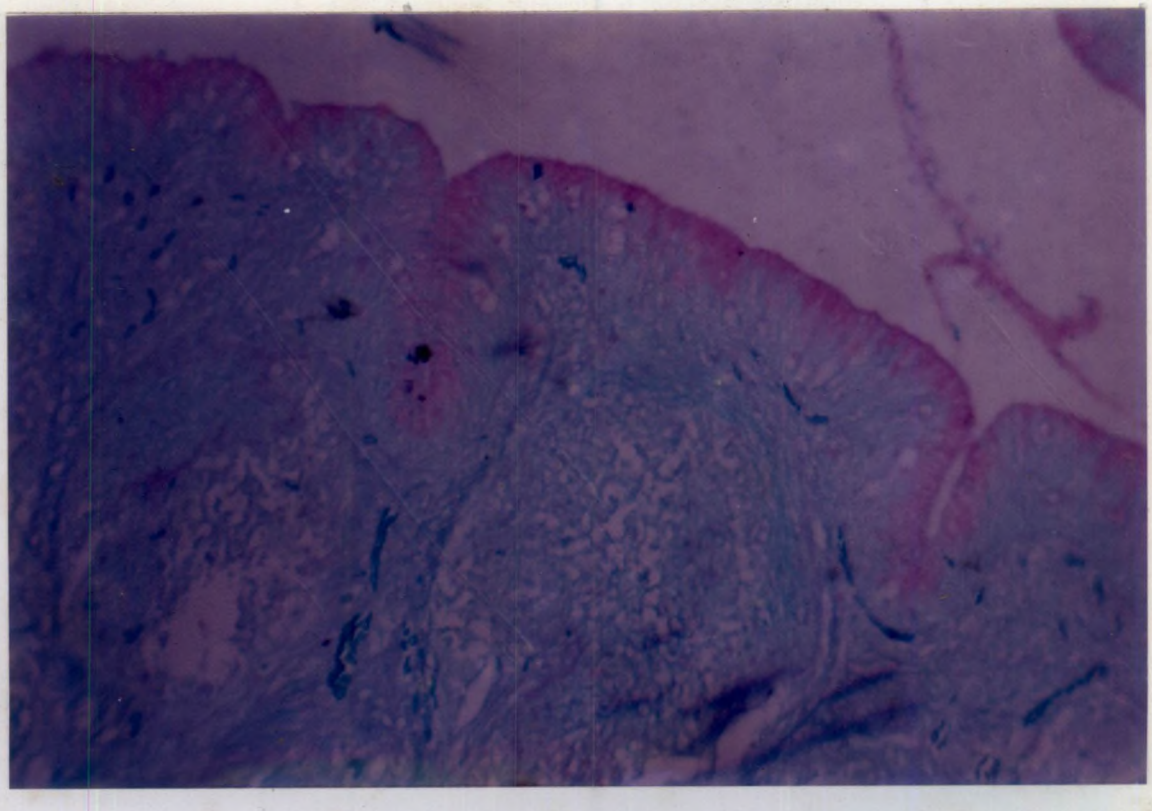


Fig.35. Section of bursa showing PAS positive reaction in the epithelium lining tunica mucosa (28 days)

PAS method x 160

Fig.36. Section of bursa showing metachromasia of lining epithelial cells (35 days)

Toulidine Blue method x 160



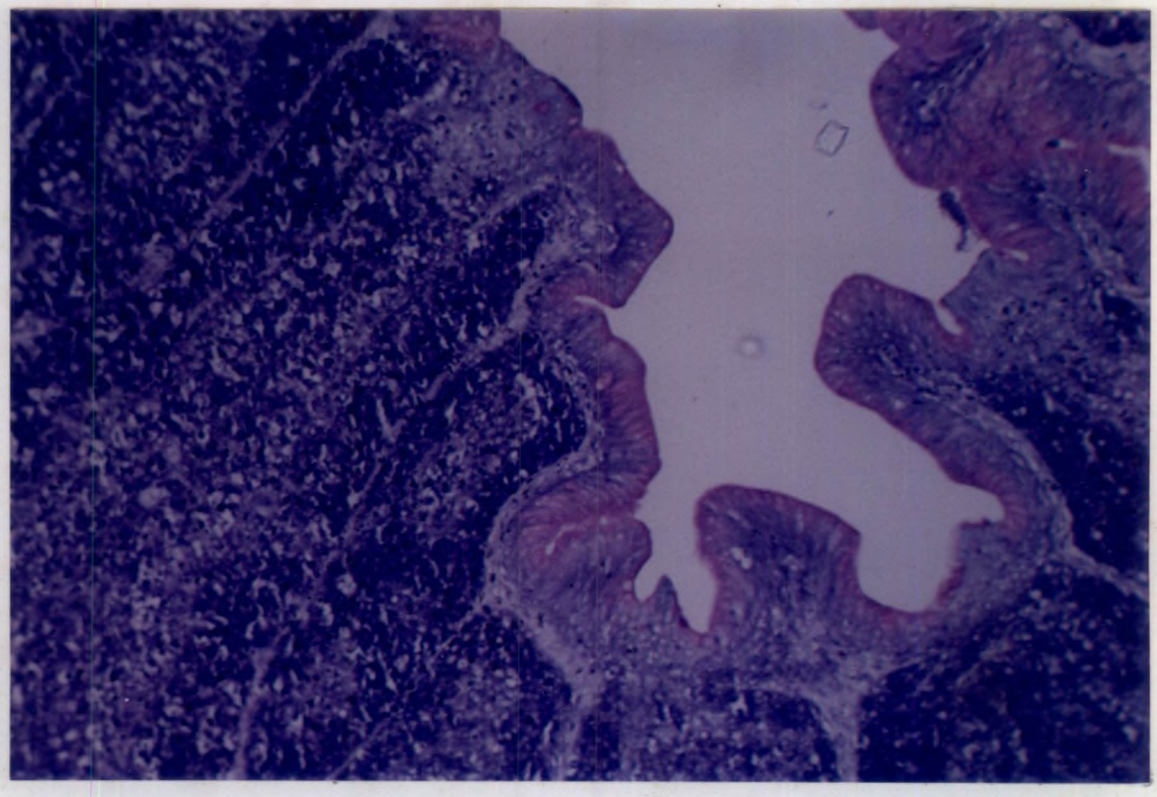
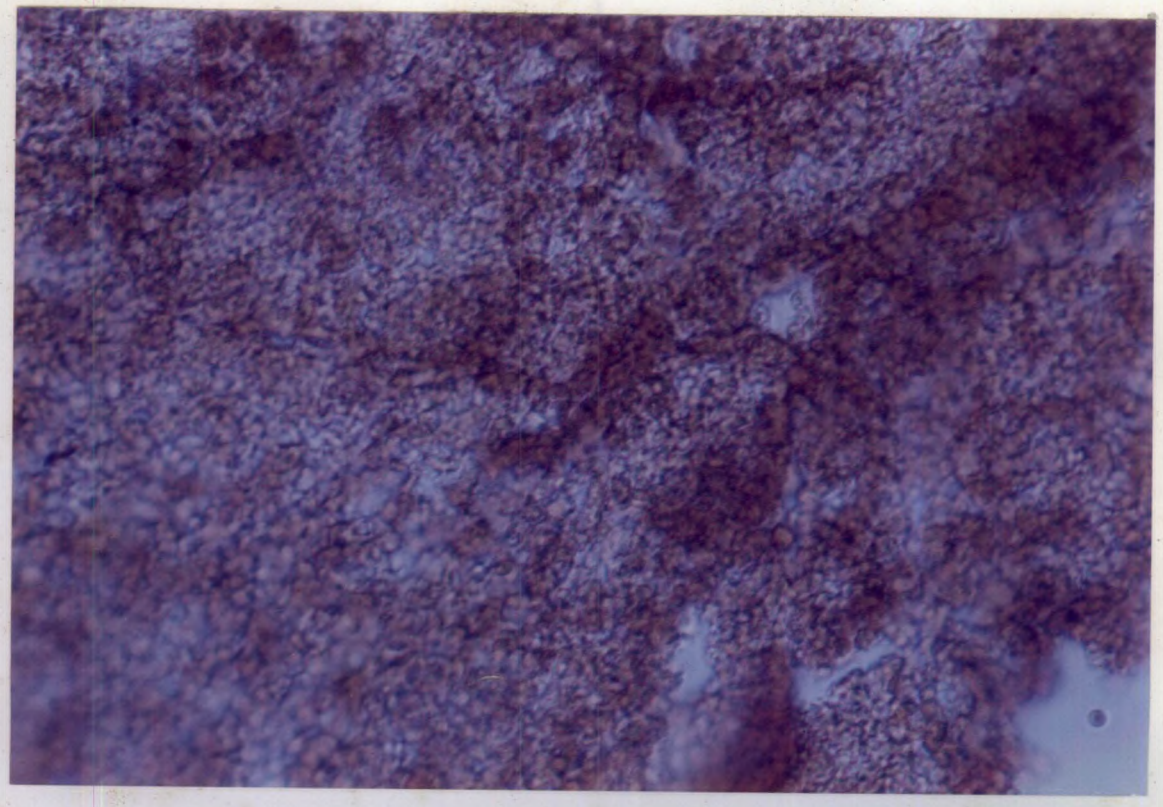


Fig.37. Section of bursa showing moderate acid phosphatase activity in the lymphoid follicles (35 days)

Napthol AS-B_1 Phosphate method x 250

Fig.38. Section of bursa showing mild alkaline phosphatase activity in the lining epithelium and follicles (58 days)

Modified Gomori's method x 250



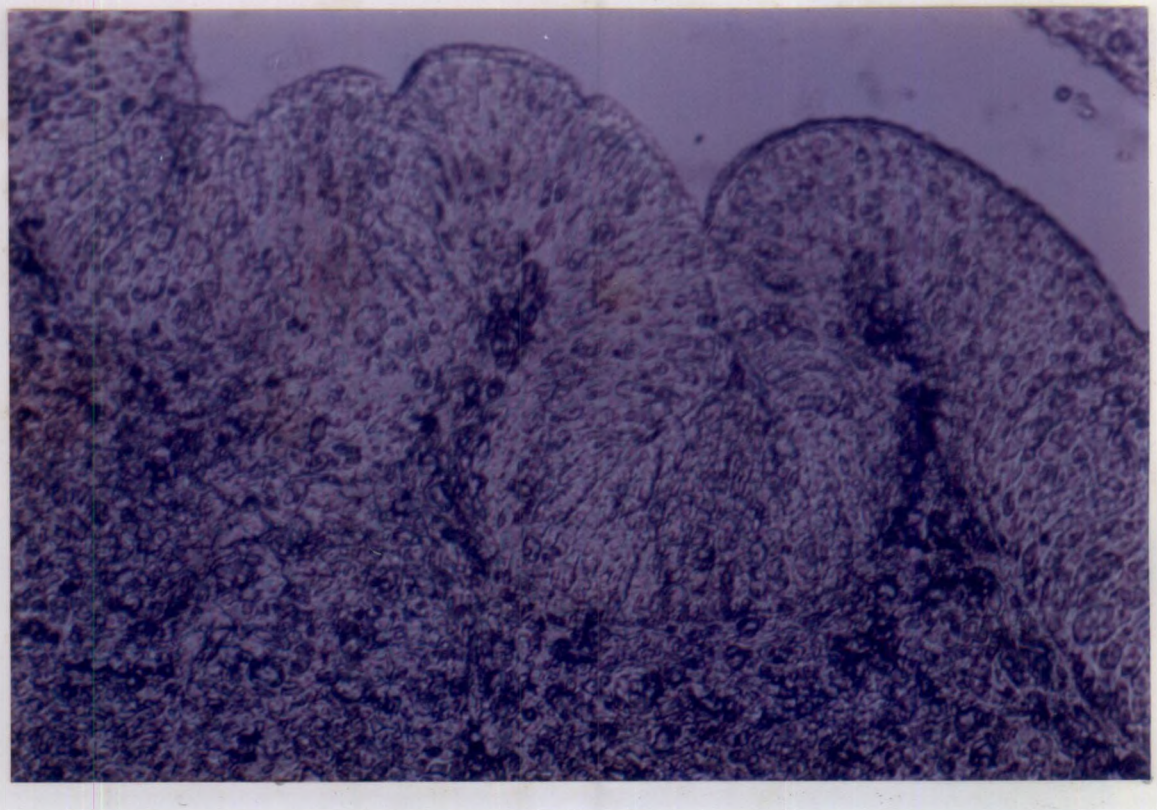
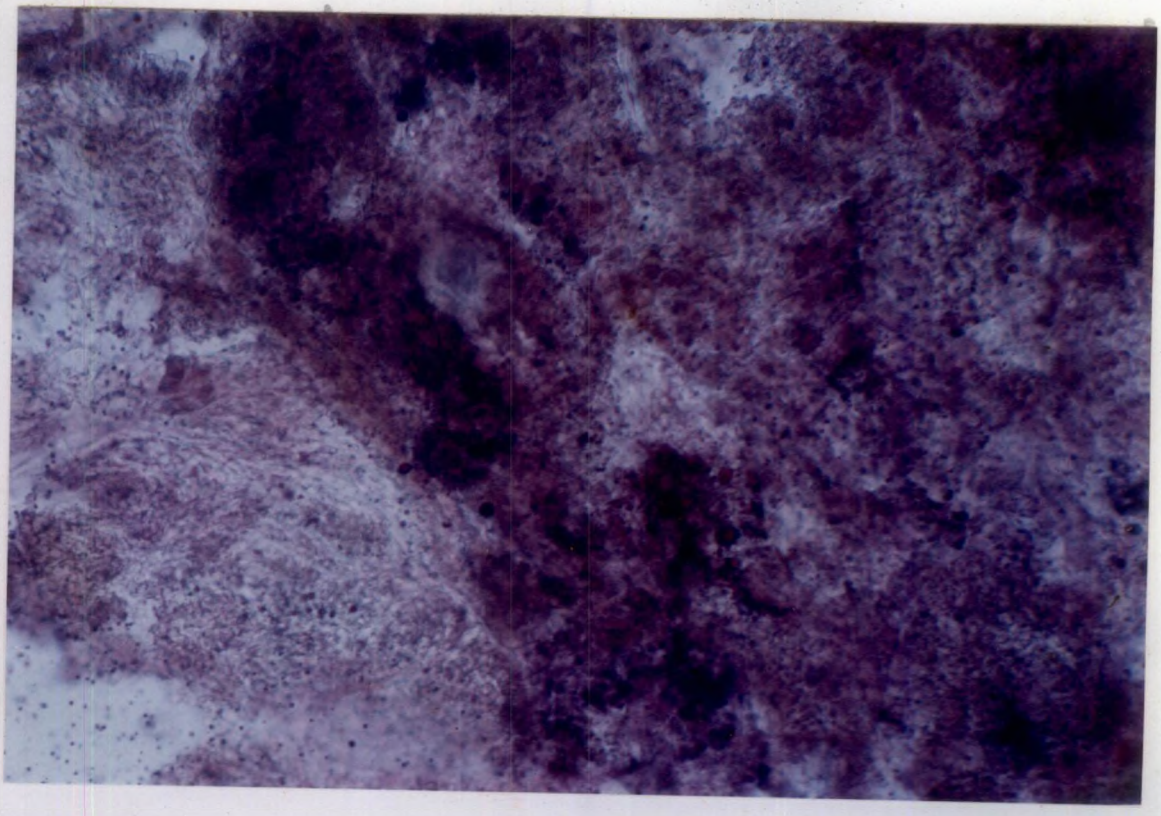


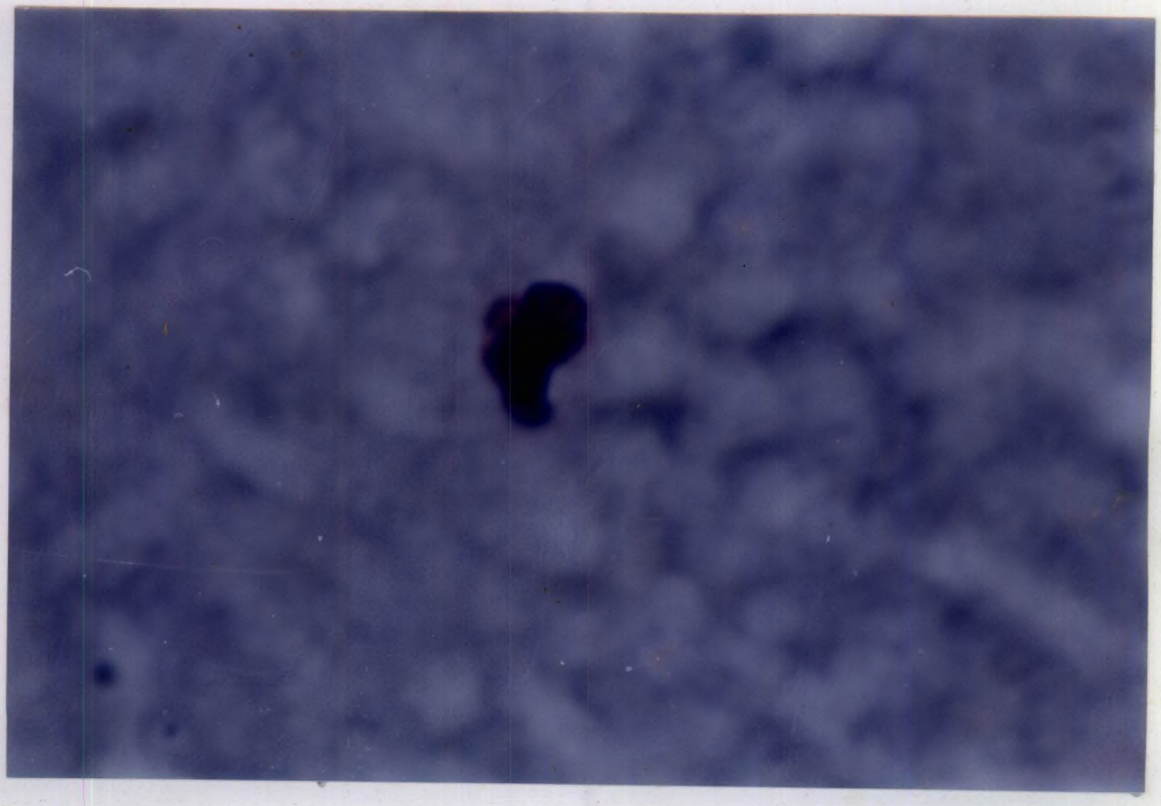
Fig.39. Section of bursa showing lipid accumulation in the propria mucosa

Oil Red `O' in propylene glycol method x 250

Fig.40. Immunoperoxidase staining of bursal impression smear showing brown precipitate at the site of antibody localisation (50 days)

Immunoperoxidase method x 1000





Discussion

5. DISCUSSION

The bursa of Fabricius in the day old White Pekin ducklings occurred as an elongated blind sac like structure with a tapering apex. Similar observations have been reported by Hashimoto and Sugimura (1976) in White Pekin ducklings and Chakravarthy and Sastry (1982) in local ducks (*Anas boscas*). This contrasts with the shape of the chicks bursa which is round or oval blind sac like structure (Glick, 1963). The bursa communicated with the dorsal proctodeal region of cloaca by a short stalk as reported earlier in various avian species (Payne, 1971; Glick, 1980, Chakravarthy and Sastry, 1982).

In all the young ones, up to 20 days of age the bursa was cylindrical and caecum like with tapering apex and by 58 days the bursa was cylindrical with pointed end. Thereafter by 155 days the bursa was cylindrical and much reduced in size. Contrary to this Chakravarthy and Sastry (1982) reported that in 'Desi' ducks (*Anas boscas*) the bursa was cylindrical with a tapering end up to two months, cylindrical with a pointed end by four months and thereafter by 6 months the bursa was cylindrical, little longer and less in circumference with a pointed end. These variations might be attributed to the breed difference.

The colour of bursa was pale yellow up to 20 days, yellowish white by 58 days and pale white by 155 days. Similar reports in the duck are not available for comparison.

In the day old ducklings the inner luminal surface showed about six small folds which increased to eight by 10 days of age and ten by 30 days of age. After 95 days of post natal life, the plicae count decreased to eight. This was suggestive of commencement of regressive changes in the bursa. At 155 days of age only six plicae could be counted. Similar reports regarding the changes in the number of plicae in growing ducklings are not available for comparison. In the lumen of the bursa 12-14 longitudinal folds were recorded in ducks (Chakravarthy and Sastry, 1982) and chicken (Ackerman and Knouff, 1959; Hodges, 1974).

Among the primary plicae in the inner luminal surface of the bursa two of the folds located on the ventral aspect were longer and well developed in birds of all age groups. A similar observation was made by Chakravarthy and Sastry (1982) in ducks. In contrast the luminal surface of the bursa of chicken contained longitudinal folds of same length all around its circumference (Ackerman and Knouff, 1959).

The proportion of bursa weight to body weight percentage showed a decreasing trend at all ages indicating that after hatch the bursal growth rate was not in proportion to the body growth rate. The whole body grew at a faster rate compared to the bursa. An observation similar to this was made by Hashimoto

and Sugimura (1976) in White Pekin ducks. On the day of hatch the mean weight of the bursa represented 0.23 per cent of the gross body weight and reached its maximum weight relationship (0.49 per cent) to the body at 5 days of age and thereafter grew less rapidly than the body At 58 days of age, when the bursa showed its mean peak bursal weight it represented 0.17 per cent of body weight. At 155 days the mean bursal weight represented only 0.05 per cent of the body.

Wolfe *et al.* (1962) recorded that in chicken the maximum mean weight relationship of bursa to the body was achieved at four weeks. At 10 weeks of age when the bursa reached the maximum mean weight it represented 0.3 per cent of body weight. The mean bursal weight at 23 weeks of age represented only 0.014 per cent of the body weight. These variations from the present results might be due to the species difference. King (1975) reported that the weight of the bursa of ducks as a percentage of body weight was 0.13 per cent on the day of hatch and 0.02 per cent at 22 weeks of age. Hashimoto and Sugimura (1976) recorded that at one week of age the bursa reached its maximum weight relationship (0.42%) to the body. At 9 weeks when the bursa attained its maximum weight it represented only 0.09 per cent of body weight. These slight variations might be due to the differences in the managemental practice.

The datas collected by Glick (1956) using three different breeds of chicken showed a mean peak bursal weight at eight weeks for male Rhode Island Red, four weeks for male White Leghorn and eight to fourteen weeks for males hatched

from a cross between Barred Plymouth Rock and Dominant White Rocks. Wolfe *et al.* (1962) reported that in the Arbor Acre White Rock the bursa showed the most rapid growth in the first four weeks after hatch and reached its maximum mean weight at the tenth week which was followed by a steady regression upto 23 weeks of age. Romppanen (1982) described that in White Leghorn chicken at 9 weeks age maximum mean absolute weight was achieved.

In Japanese quails, as reported by Yamada *et al.* (1973), the bursa attained its mean maximum weight at 7 weeks in the males and at 6 weeks in females.

In White Pekin ducks, Glick (1960) reported that the bursa attained its maximum weight at 8 weeks of age. Hashimoto and Sugimura (1976) noted that there was a rapid growth in bursa of White Pekin ducks during the first 5 weeks after hatch and it reached its maximum weight at 9 weeks of age which was followed by a rapid decrease in weight.

In the present study the bursa showed the most rapid growth in the first 28 days after hatch and reached its maximum weight at 58 days of age, which was followed by a gradual decrease upto 155 days of age. As compared with the data in chicken by Glick (1956), Wolfe *et al.* (1962) and Rompannen (1982), in ducks the bursa reached the mean maximum weight at an earlier age with the exception of the data for male White Leghorn given by Glick (1956). The period of most rapid growth was similar in both the species. When compared with the data of quail by Yamada *et al.* (1973), the duck's bursa reached its mean maximum weight at a later stage of life. The slight variations in the age of maximum mean bursal weights and most rapid growth of bursa in the present results from the previous reports on

White Pekin ducks by Glick (1960) and Hashimoto and Sugimura (1976) might be due to differences in the managemental practices.

It was observed that both age and body weight influenced the weight of bursa. A highly significant positive correlation was recorded between age and body weight, bursa weight and body weight as well as bursa weight and age. Since a greater percentage of the total variation in bursa weight was accounted for by body weight when the age of the bird was kept constant, it was inferred that heavier birds of a particular age would tend to have heavier bursa. These observations tally with the findings of Chakravarthy and Sastry (1982) in ducks.

The length and diameter (top and bottom) of bursa showed an increasing trend with the advancement of age upto 58 days. This was followed by a steady decrease upto 155 days. A similar growth pattern was reported in White Pekin ducks by Hashimoto and Sugimura (1976). However, in local duck (*Anas boscas*) (Chakravarthy and Sastry, 1982) a gradual increase in length of bursa upto 6 months and diameter of bursa upto 4 months of age was recorded. The diameter of bursa decreased thereafter upto 6 months. These differences in results might be due to breed variation.

The histological structure of the day old and 155 days old bursa was similar except for variations in size of the plicae, the lining epithelium of bursal mucosa, lymphoid follicles and number of lymphocytes in it. In Japanese quails, Basha (1993) recorded that the histological structure of the day old and second week

bursa was similar to the sixteenth day embryonic bursa except for the increase in the size of the lymphoid follicles, with clearly demarcated cortex and medulla which in turn increased the height and width of the plica.

The division of the wall of the bursa into three tunics - the outer serosa, covering the entire organ middle muscularis and an inner mucosa; concur with those of other avian species (Payne, 1971; Hodges, 1974; Firth, 1977; Basha, 1993).

In the tunica muscularis; outer circular and inner longitudinal layer of smooth muscle fibres recorded in the 30 days old bursa concurs with the findings in turkey (Malewitz and Calhoun, 1958) and quails (Basha, 1993). However, Hodges (1974) and Firth (1977) reported that the muscle layers tended to be very variable in chicken. At closely adjacent points on the circumference of the gland the muscularis had the appearance of a single longitudinal layer, an outer longitudinal layer and an inner circular layer, or two longitudinal layers with an interposed circular layer. Thus, at least two muscle layers were seen running obliquely or at right angles to each other. In White Pekin ducks, Hashimoto and Sugimura (1976) reported that the outermost part of the bursa in cross section consisted of a circular strand of smooth muscle and serosa.

In the day old bursa, the two muscle layers were very thin and could not be delineated. However, with advancement in age a gradual increase in thickness of

muscle fibres was reported. Similar reports in ducks are not available for comparison.

Presence of blood vessels and its branches between the muscle layers and in the corium of each plica noticed here concurs with the findings in chickens (Hodges, 1974; Firth, 1977) and quails (Basha, 1993).

The tunica mucosa consisting of a lining epithelium and lamina propria filled with lymphoid follicles observed in the present study confirmed the earlier findings in other avian species (Malewitz and Calhoun, 1958; Hodges, 1974; Firth, 1977; Glick, 1983; Basha, 1993) including ducks (Hashimoto and Sugimura, 1976; Chakravarthy and Sastry, 1982).

The bursal mucosa in ducks was thrown into folds, the primary plicae each of which branched to form secondary plicae as reported earlier in chickens (Hodges, 1974; Glick, 1983, Thandavamoorthy, 1989) quails (Yamada *et al.*, 1977; Basha, 1993) and ducks (Chakravarthy and Sastry, 1982). According to Glick and Olah (1982) such plicae are not evident in the European starling.

The increase in height and width of the primary plicae paralleled the growth of the bursa and the follicles in it upto 58 days of age. Thereafter, a gradual decrease was recorded upto 155 days post hatch. Similar reports in the duck are not available for comparison. In chicken (Payne, 1971;

Thandavamoorthy, 1989) and quails (Yamada *et al.*, 1977; Basha, 1993) the plicae increased in height and diameter concommitant to the growth of lymphoid follicles upto the age of sexual maturity.

The pseudostratified columnar epithelium lining the bursal mucosa observed in the present study was in total agreement with the findings of Hodges (1974) and Firth (1977) in chickens. However in turkey (Malewitz and Calhoun, 1958) and in quails (Basha, 1993) the lining epithelium was pseudostratified columnar at the plical tip and at certain folds between the plica it was simple columnar. These findings are contrary to the observations made by Chakravarthy and Sastry (1982) who reported that the lining epithelium of the bursa was columnar in local ducks (*Anas boscas*).

The average height of columnar epithelial cells increased upto 35 days of age and thereafter remained constant. Hashimoto and Sugimura (1976) made similar reports in ducks. According to them the epithelium reached its maximum height at 5-7 weeks and initial regressive changes commenced from 13th week only. Similarly in the present experiment also the epithelium showed degenerative changes from 95 days of age onwards.

In birds of all ages the bursal epithelium exhibited positive reaction to PAS indicating the presence of mucopolysaccharides. This concurs with the findings in chicken (Ackerman and Knouff, 1959; Hodges, 1974; Thandavamoorthy, 1989). Metachromasia of the lining epithelial cells with toulidine blue detected in birds of

all age groups indicated the presence of mucin. Similar observations in ducks are not available for comparison.

The bursal epithelium revealed intense acid phosphatase activity and moderate alkaline phosphatase activity in birds of all age groups. Concurrent reports were made by Fennel and Pearse (1961) in chicken and Basha (1993) in quails. A positive reaction of the bursal epithelium for PAS and toulidine blue along with intense acid phosphatase activity is an indication of the presence of glycoprotein which are the basis for antibodies (Basha, 1993).

Three types of cells - Type I, Type II and Type III were identified in the lining epithelium of ducks bursa. Type I cell was columnar in shape with round or oval nucleus, Type II cell was columnar or low columnar cell with an oval nucleus located above or below the midline of the cell and Type III appeared to be a goblet cell. The presence of similar cells were earlier reported by Ackerman and Knouff (1959), Hodges (1974), Firth (1977) and Thandavamoorthy (1989) in chickens and Basha (1993) in quails. All the three types of cells could be identified up to 95 days of age only. Similar reports regarding the exact age after which the cells could not be delineated are not available for comparison.

The epithelium was distinguished into two alternating areas, the follicle associated epithelium (FAE) showing pale columnar cells and interfollicular epithelium (IFE) showing darkly stained columnar cells. This difference in epithelial structure is in accordance with the findings in ducks (Hashimoto and

Sugimura, 1976) and in chickens (Hodges, 1974; Thandavamoorthy, 1989). In the IFE cells, degenerative changes were recorded from 42 days of age but there was no change in the FAE cells until 95 days of age. These findings agree with those reported by Hashimoto and Sugimura (1976) in White Pekin ducks. The FAE cells are reported to play a major role in interacting with the antigenic material coming in contact with its surface as it possesses pinocytic capacity (Olah and Glick, 1978; Dellman, 1993).

The inter follicular connective tissue was made of a network of fine collagen, reticular and a few elastic fibres. Large blood vessels were also found in the interfollicular connective tissue. Similar observations were made by Hashimoto and Sugimura (1976) in ducks, Hodges (1974) and Firth (1977) in chicken and Basha (1993) in quails.

Eosinophils and plasma cells were noticed in the interfollicular and subepithelial connective tissue. The maximum number of plasma cells were recorded at 20-30 days of age, which is in total agreement with the findings of Hashimoto and Sugimura (1976) in ducks and Basha (1993) in quails. The existence of greater number of plasma cells in the interfollicular space of the bursa at three weeks of age might be suggestive of the beginning stage of active antibody production in ducks, and the ability might start from the second or third week of the post hatch life to counterbalance the fall in serum gamma globulin

acquired earlier from the maternal bird as reported by Hashimoto and Sugimura (1976).

Mast cells, macrophages and undifferentiated epithelial cells were also observed in lamina propria as reported earlier by Naukkarinen *et al.* (1982) in chicken and Basha (1993) in quails.

The greater portion of the space within each plica was occupied by the lymphoid follicles which were spherical in day old ducklings but assumed an elongated outline by 20 days of age. An observation similar to this was made in chicken (Bradley, 1960) and duck (Hashimoto and Sugimura, 1976).

The number of bursal lymphoid follicles of the duck was almost fixed from the day of hatch up to 65 days of age and decreased thereafter. But Hashimoto and Sugimura (1976) opined that the follicle number was almost fixed up to 13 weeks in ducks. Yamada *et al.* (1977) and Basha (1993) reported an increase in number of follicles during the first five weeks of post hatch period in the bursa of Japanese quails. The number of follicle counted per cross section of duck bursa did not correspond to the number recorded by Hashimoto and Sugimura (1976) in ducks.

The increase in diameter of the bursal lymphoid follicles up to 58 days of age followed by a decrease thereafter, observed in the present study confirmed the reports of Hashimoto and Sugimura (1976) in ducks who recorded a steady

increase in size of follicles up to ninth week followed by a gradual decrease thereafter and Ibragimov (1976) who reported maximum follicle diameter at day 65 in chicken. However, Yamada *et al.* (1977) reported a rapid increase in size of the follicles during the first five weeks of post hatch period, in quails.

In the present study, the size of lymphoid follicles changed with age and coincided with the weight of the bursa. These tally with the observations made by Hashimoto and Sugimura (1976) in ducks and Yamada *et al.* (1977) in quails.

Lymphoid follicles containing a central medulla and peripheral cortex were clearly demonstrable in the day old ducklings which concurs with the results of Hashimoto and Sugimura (1976) in ducks. Whereas Ibragimov (1976) stated that the cortex and medulla of the lymphoid follicle were well distinct only at 75 day of age in chicken. According to Thandavamoorthy (1989) a well defined cortex could be identified only at 15 days post hatch in chicken. Similarly in quails, Basha, (1993) reported that the cortex and medulla were clearly demonstrable only at 15 days of age. This observation that the lymphoid follicles in the bursa of ducks were more functional at the time of hatch as compared to the quail and chick bursa, indicated an early development of immunocompetence in ducks.

The cortex contained densely packed lymphocytes supported by a mesenchymal reticular network and capillaries. The inner medulla contained loosely packed large lymphocytes in the periphery and small lymphocytes in the centre. The cortex is separated from the medulla by a thin layer of

undifferentiated epithelial cells with distinct basement membrane and a thin layer of capillary network. The undifferentiated epithelial cell layer with basement membrane was in continuity with the basement membrane and surface epithelium of the plica. These findings are in agreement with the reports of Payne (1971), Glick (1983), Thandavamoorthy (1989) and Dellmann (1993) in chicken and Hashimoto and Sugimura (1976) in ducks. Occurrence of other cells such as macrophages, and reticular cells recorded in the medulla of bursal follicle is as reported earlier in chicken by Frazier (1974) and Naukkarinen *et al.* (1982). Contradictory reports regarding the lymphoid cellular content of the cortex and medulla were made by Chakravarthy and Sastry (1982) in ducks. They recorded only closely packed small lymphocytes in the cortex and loosely packed large lymphocytes in the medulla up to six months of age. However, in the present study the lymphocyte population in both the cortex and medulla were predominantly of small lymphocytes from 140 to 155 days of age in ducks suggestive of marked regression of the bursa.

In the present study the lymphocytes of both the cortex and medulla of the follicle were similar in structure as observed by Clawson *et al.* (1967) in chicken but is contrary to the findings of Casali *et al.* (1977) who stated that the medullary lymphocytes of the bursal follicle in chicken were homogenous while cortical cells were heterogeneous in appearance.

The maximum number of immuno-peroxidase positive cells were recorded from 35 to 58 days of age indicating that the stage of active antibody production in ducks coincides with the age at which maximum number of plasma cells and lymphocytes were detected in the bursa. Similar reports regarding the bursa of duck are not available for comparison.

The width of cortex and diameter of medulla showed a gradual increase corresponding to the increase in follicular diameter and showed maximum size at 58 days of age which was followed by a gradual reduction in size up to 145 days and a tremendous drop there after up to 155 days. The lymphocyte population per follicle also followed a similar trend. Maximum number of lymphocytes were observed in 58 day old bird and decreased thereafter. These observations indicated that the bursa was at its peak functional activity at an age of 58 days. Similar observations regarding width of cortex and diameter of medulla in ducks are not available for comparison.

In White Pekin ducks the bursa showed mild regressive changes from 95 days, but very prominent microscopic features of involution were evident from 140 days of age only. Similarly, Hashimoto and Sugimura (1976) reported that initial histological changes in the bursa during its natural regression were found in the 13 week old ducks.

The histological changes in the bursa of chicken during its natural regression reported by Bickford *et al.* (1985) were follicular atrophy, depletion of cortical lymphocytes, thinning, vacuolation, folding and detachment of plical and interplical epithelium. Similar changes were noticed in the bursa of ducks also in the present experiment but at an earlier age of 140 days as against 24 weeks in White Leghorn chicken. The cysts observed by Hashimoto and Sugimura (1976) in the bursa of ducks and Bickford *et al.* (1985) in bursa of chickens during its natural regression were not observed in the present study. Scala *et al.* (1988) reported that cysts were not observed in the involuted bursa of ducks. Invasion of adipose tissue into the propria was noticed at 155 days of age in the involuted bursa of ducks in this study is in agreement with the findings of Bickford *et al* (1985) in chickens, Hashimoto and Sugimura (1976) in ducks and Yamada *et al.* (1977) and Basha (1993) in quails.

The earliest involutary changes characterized by atrophy and detachment of interplical and plical surface epithelium and lysis of follicular lymphocytes, suggested an abrupt interruption of bursal homeostasis. The intermediate and terminal stages of involution represented progressively more advanced reaction to the presence of necrotic material in the bursal mucosa - i.e., macrophagic phagocytosis, fibroplasia, and ultimate fibrous replacement of space once occupied by lymphoid follicles is as reported by Bickford *et al.* (1985) in chicken.

The bursa of Fabricius involutes concomitantly with the development of adrenal gland (Glick, 1960) and functional gonads and changes paralleling involution were induced by natural or synthetic gonadal hormones (Payne, 1971; Firth, 1977) cortisone and corticosterone (Dieter and Breitenbach, 1970) ACTH (Sato and Glick, 1965). These hormones effect bursal homeostasis and induce regressive changes in the bursa.

Summary

6. SUMMARY

Post natal development of the bursa of Fabricius in the White Pekin ducks was studied using 51 ducks of day-old to 155 days of age. In the day-old ducklings, the bursa could be seen as a yellow, smooth, elongated blind sac-like structure with a tapering apex. It communicated with the proctodeum of the cloaca dorsally by a short stalk, in birds of all ages. By 58 days the bursa was cylindrical with pointed apex and yellowish white in colour. Thereafter by 155 days the bursa was a cylindrical and much reduced pale structure.

The inner surface of the bursa was plicated containing two large well developed longitudinal folds on the ventral aspect and about five to eight smaller folds all around the circumference. The number of plicae increased upto 30 days of post natal life and remained constant till 80 days of age. By 95 days a decreasing trend was recorded and the 155 day old bursa had only six plicae. The plical measurements length and diameter of bursa showed an increasing trend upto 58 days of post hatch period and declined thereafter.

The proportion of bursal weight to body weight percentage showed a decreasing trend at all ages indicating that after hatch the bursal growth rate was not in proportion to that of body. The bursa reached its maximum mean weight relationship (0.49%) to the body at five days of age.

The maximum mean weight of bursa was noted at 58 days of age. At 155 days of post natal period, the contribution of bursa to body weight was only 0.05 per cent. Highly significant positive correlation was observed between age and body weight as well as the bursal weight and body weight.

It was observed that 72.2 per cent of the total variation in bursal weight was explained by the variation in the age and body weight of the bird. When body weight of the bird was kept constant 47.6 per cent of the total variation in the bursal weight was accounted for by age. When age of the bird was kept constant 62.4 per cent of the variation in weight of the bursa was accounted for by body weight. Hence, the body weight had a greater influence on the weight of the bursa.

The gross as well as biometric observations in the study indicate that in ducks bursa may be most functional by 58 days post hatch.

In birds of all ages, histologically the wall of the bursa was divided into three tunics - the outermost tunica serosa, the middle tunica muscularis and the innermost tunica mucosa.

The serosa was thin and enveloped the entire organ. It increased in thickness gradually as age advanced.

The tunica muscularis consisted of an outer circular and inner longitudinal layer of smooth muscle fibres with blood vessels in between. The two muscle layers could be delineated clearly by 30 days of age only.

The tunica mucosa, consisted of a lining epithelium and lamina propria filled with follicles in birds of all ages. Upto 95 days of age the pseudostratified columnar lining epithelium of bursa distinctly identified the three cell types - columnar, low columnar and goblet cells. The lymphoid follicular relationship of this lining epithelium distinguished it into follicle associated epithelium (FAE) and interfollicular epithelium (IFE). Though mild degenerative changes were recorded in the IFE cells from 42 days of age, there was no change in the FAE cells until 95 days of age after which the degenerative changes in it lead to spillage of lymphocytes into the lumen from degenerating follicles.

Immunologically potent part of bursa - the lymphoid follicles were packed in the lamina propria. Each follicle consisted of a cortex and medulla separated by a layer of undifferentiated epithelial cells with distinct basement membrane and a thin layer of capillary network in birds of all ages.

The cortex of the follicles contained densely packed lymphocytes, lymphoblasts, plasma cells, few macrophages, many mitotic figures and fine arterioles. In the medulla loosely arranged lymphocytes, reticuloepithelial cells and macrophages were recorded in birds of all age groups.

Large lymphocytes predominated in the cortex upto 58 days of age. Thereafter, the small lymphocytes were comparatively more in the follicular cortex. In the medulla, large lymphocytes were detected near the cortico medullary junction and loosely arranged small lymphocytes occupied the central region of lymphoid follicles. By 155 days only small lymphocytes could be located in the follicles.

The observations on the number, size, cellular details and corticomedullary differentiation of lymphoid follicles indicated that they were most functional by about 58 to 65 days of post hatch period in White Pekin ducks.

The cellular component of lamina propria also contained plasma cells, eosinophils, mast cells, macrophages and fat cells. Adipose cells increased as age advanced to mark the regression of this lymphoid organ. The interfollicular and subepithelial connective tissue was made up of a network of fine collagen fibres, few elastic fibres and numerous reticular fibres with blood vessels at the base or centre of each plica. The connective tissue increased tremendously in the 155 day old bursa.

During its natural regression, the bursa showed follicular atrophy, fibrosis of subepithelial stroma, plical fusion and depletion of cortical lymphocytes. Further, thinning, vacuolation, folding and detachment of plical and interplical epithelium were also evident. By 155 days, the mucosal remnants in the bursa

consisted of numerous coalescing degenerating follicles intermingled with fibrous connective tissue and adipose tissue.

The lining epithelial cells of the bursal mucosa revealed positive reaction to Schiff's reagent and metachromasia in birds of all ages. Intense acid phosphatase and moderate alkaline phosphatase activities were also noticed in tunica mucosa of the bursa, in birds of all age groups.

The maximum positive immunoperoxidase activity could be seen by about 35 to 58 days of age. This coincided with micrometric and other biometric observations.

It can be concluded that in the bursa of ducks, the immunological functions commenced from the fourth or fifth week in the postnatal life and attained its peak functional activity by two months of age. The morphometric changes in bursa with age was mainly influenced by the micrometric changes in the lymphoid follicles.

References

REFERENCES

Ackerman, G.A. and Knouff, R.A. (1959). Lymphocytopoiesis in the bursa of Fabricius. *Am. J. Anat.*, **104**(2): 163-178.

Ackerman, G.A. and Knouff, R.A. (1964). Lymphocytopoietic activity in the bursa of Fabricius: *The Thymus in Immunobiology*. Edited by Good, R.A. and Gabrielson, A.E., Hoeber Medical Division, Harper and Row Publishers, New York, pp. 123-146.

*Adelmann, H.B. (1967). *The Embryological Treatises of Hieronymus Fabricius of Aquapendente*, Cornell University Press, Ithaca, New York.

Bancroft, J.D. and Stevens, A. (1977). *Theory and Practise of Histological Techniques*, Churchill Livingstone, Edinburgh, pp. 148-149.

Barka, T. (1960). A simple azo dye method for histochemical demonstration of acid phosphatase. *Nature*, **187**: 248.

Basha, S.H. (1993). *Histomorphology and histochemistry of the thymus and the bursa of Fabricius in Japanese quail(Coturnix coturnix japonica)*. M.V.Sc. thesis submitted to TANUVAS, Madras.

Bickford, A.A., Kuney, D.R., Zander, D.V. and McMartin, D.A. (1985). Histological characterisation of the involuting bursa of Fabricius in single comb White Leghorn chicks. *Avian Dis.* **29**(3): 778-797.

Bradley, O.C. (1960). *The structure of the fowl*, Oliver and Boyd, Edinburgh, London, 4th Ed., pp. 28-35.

Calhoun, M.L. (1954). *Microscopic anatomy of the digestive system of the chicken*, Iowa State College Press,Ames, Iowa. pp. 55-60.

*Casali, A.M., Grossi, C.E., Guidothi, L.M. and Marzoli, F.A. (1977). Cytophotometric characterisation of cortical and medullary lymphocytes in chicken bursa and thymus. *Beitrage Zur Pathologic.*, **160**(3): 231-244.

Chakravarthy, Y.S. and Sastry, A.P. (1982). Bursa of Fabricius in local ducks (*Anasboscas*). *Indian Vet. J.* **59**(1): 64-66.

Chang, T.S., Rheins, M.S. and Winter, A.R. (1957). The significance of the bursa of Fabricius in antibody production in chickens. I. Age of chickens. *Poult. Sci.*, **36**(4): 735-738.

Clawson, C.C., Cooper, M.D. and Good, R.A. (1967). Lymphocyte fine structure in the bursa of Fabricius, the thymus and the germinal centres. *Lab. Invest.* **16**: 407421.

*Davy, J. (1866). On the bursa of Fabricii. *Proc. Roy. Soc. Lond.* **15**: 94-102.

Deiter, M.P. and Breitenbach, R.P. (1970). The growth of chicken lymphoid organs, testes and adrenals in relation to the oxidation state and concentration of adrenal gland and lymphoid organ. *Poult. Sci.* **47**: 1463-1469.

Dellmann, H.D. (1993). Lymphatic system: *Textbook of Veterinary Histology*, Lea and Febiger, Philadelphia, 4th Ed., pp. 280-282.

Dominic, C.J. (1962). Some remarks on the follicles of the bursa of Fabricius in birds. *Science Culture (India)***28**: 20-21.

Drury, R.A.B. and Wallington, E.A. (1980). *Carleton's Histological Technique*, Oxford University Press, Oxford, 5th Ed., pp. 51-52, 196-197.

Fennel, R.A. and Pearse, A.G.E. (1961). Some histochemical observations on the bursa of Fabricius and thymus of the chicken. *Anat. Rec.* **139**: 93-103.

Firth, G.A. (1977). The normal lymphatic system of the domestic fowl. *Vet. Bull.* **47**(3): 167-179.

*Forbes, W.A. (1877). On the bursa of Fabricii in birds. *Proc. Zool. Soc. Lond.* 1877: 304-318.

Frazier, J.A. (1974). The ultrastructure of the lymphoid follicle of the chick bursa of Fabricius. *Acta. Anat.* **88**: 383-397.

*Giurgea, R. (1977). Developmental aspects of bursa of Fabricius and thymus during post-hatching ontogenesis in *Gallus domesticus*. *Annales-de-Biochemic-Biophysique* **17**(2): 173-178.

Glick, B. (1956). Normal growth of the bursa of Fabricius in chickens. *Poult. Sci.* **35**: 843-851.

Glick, B. (1960). Growth of the bursa of Fabricius and its relationship to the adrenal gland in the White Pekin duck, White Leghorn, outbred and inbred New Hampshire. *Poult. Sci.* **39**: 130-139.

Glick, B. (1963). The effect of surgical and chemical bursectomy in the White Pekin duck. *Poult. Sci.* **42**(2): 1106-1112.

Glick, B. (1980). The thymus and bursa of Fabricius. Endocrine Organ: *Avian Endocrinology*. Academic Press Inc., New York, pp. 209-229.

Glick, B. (1983). Bursa of Fabricius. *Avian Biology*. Vol. VII. Edited by Farner, D.S., King, J.R. and Parkes, K.C. Academic Press, New York.

Glick, B. and Olah, I. (1982). The morphology of the starling (*Sturnus vulgaris*) bursa of Fabricius: A scanning and light microscope study. *Anat. Rec.***204**: 341-348.

Gridley, M.F. (1951). A modification of the silver impregnation method of staining reticular fibres. *Am. J. Clin. Path.* **21**: 897-899.

Hashimoto, Y. and Sugimura, M. (1976). Histological and quantitative studies on the post natal growth of the thymus and the bursa of Fabricius of White Pekin ducks. *Jap. J. Vet. Res.* **24**: 65-76.

Hinshaw, W.R. (1953). The bursa of Fabricius. *Vet. Med.* **48**: 164.

Hodges, R.D. (1974). *The histology of the fowl*. Academic Press, London. pp. 206-207.

*Ibragimov, V.A. (1976). Morphogenesis of the bursa of Fabricius in fowl. *Moskovskaya Veterinaranya* **85**: 40-42.

Islam, M.R., Nessa, J. and Halder, K.M. (1993). Detection of duck plague virus antigen in tissues by immunoperoxidase staining. *Avian Pathology* **22**: 389-393.

*Jolly, J. (1915). La bourse de Fabricius et les organes lympho-epitheliaux. *Arch. Anat. Microsc. Morphol. Exp.* **16**: 363-347.

King, A.S. (1975). Aves lymphatic system. *Sisson and Grossman's the Anatomy of the Domestic Animals*. Edited by Getty, R. Vol.2 W.B. Saunders Company, Philadelphia, London, Toronto. 5th Ed. pp. 2010-2018.

King, A.S. and McLelland, J. (1975). *Outlines of Avian Anatomy*. Balliere Tindall, London.

King, A.S. and McLelland, J. (1981). *Form and functions in birds*. Academic Press, Inc. London Ltd. pp. 352-359.

Kirkpatrick, C.M. (1944a). Body weights and organ measurements in relation to age and season in ring necked pheasant. *Anat. Rec.* **89**: 175-194.

Luna, L.G. (1968). *Manual of histologic staining methods of the Armed forces institute of Pathology*, McGraw-Hill Book company, New York, 3rd. Ed.

Malewitz, T.D. and Calhoun, M.L. (1958). The gross and microscopic anatomy of the digestive tract, spleen, kidney, lungs and heart of turkey. *Poult. Sci.* **37**: 388-398.

*Mathis, J. (1938). Zum Feinbau der Bursa Fabricii Z.Nik. *Anat. Forsch.* **43**: 179-190.

Mercer, O.S.L. and Woodward, A.E. (1987). Development of the bursa of Fabricius in the partridge and pheasant. *Poult. Sci.* **66**(3): 418-421.

Millicevic, Z., Viyic, D.c Isakovic, K., Mieic, M. and Millicevic, M. (1986). Involution of bursa of Fabricius in male and female chickens. A light microscopic histoquantitative study. *Poult. Sci.* **65**(12): 2318-2323.

Nair, G.K. (1990). *Immunoglobulins in ducks and role of bursa of Fabricius in their production.* Ph.D. thesis submitted to College of Veterinary and Animal Sciences, Mannuthy.

Nair, R.S. and Sunny, V.L. (1981). Influence of age and body weight on bursa of Fabricius in ducklings. *Cheiron* **10**(3): 140-142.

Naukkarinen, A., Arstila, A.U. and Somari, T.E. (1982). Morphological and functional differentiation of the surface epithelium of the bursa Fabricii in chicken. *Anat. Rec.* **191**: 415-432.

Naukkarinen, A. and Sorvari, T.E. (1984). Involution of the chicken bursa of Fabricius: a light microscopic study with special reference to transport of colloidal carbon in the involuting bursa. *J. Leukocyte Biology* **35**(3): 281.

Olah, I. and Glick, B. (1978). The number and size of the follicular epithelium and follicles in the bursa of Fabricius. *Poult. Sci.* **57**: 1445-1450.

Olah, I. and Glick, B. (1987). Bursal secretory cells: an electron microscopic study. *Anat. Rec.* **219**: 268-274.

Olah, I. and Glick, B. (1992). Dynamic changes in the intermediate filaments of the epithelial cells during development of the chicken's bursa of Fabricius. *Poult. Sci.* **71**: 1857-1872.

Olah, I., Glick, B., Mccorkle, F. and Stinson, R. (1979). Light and electron microscope structure of secretory cells in the medulla of bursal follicles of normal and cyclophosphamide treated chickens. *Dev. Comp. Immunol.* **13**: 101-115.

Payne, L.N. (1971). The lymphoid system: *Physiology and Biochemistry of the domestic fowl.* Edited by Bell, D.J. and Freeman, B.M. Academic Press, London. Vol.2 pp. 985-1037.

Pearse, A.G.E (1977). *Histochemistry - Theoretical and Applied.* Churchill Livingstone, Edinburgh. Vol.1, pp. 711.

Riddle, O. (1928). Growth of gonads and bursa of Fabricii in doves and pigeons with data for body growth and age at maturity. *Amer. J. Physiol.* **86**: 243-265.

*Rodak, L. and Krejci, J. (1966). Cytological and histochemical studies on the post embryonal development of the bursa of Fabricius in chicken. *Docum. Vet. Brno.* **5**: 179-188.

Romppanen, T. (1982). Post embryonic development of the chicken bursa of Fabricius: A light microscopic histoquantitative study. *Poult. Sci.* **61**: 2261-2270.

Sanjayan, K.K., Sreekumaran, T., Varhese, K., Valsala, K.V. and Ramachandran, K.M. (1996). *J. Vet. Anim. Sci.* **27**: 59-63.

Sato, K. and Glick, B. (1965). Reduction of anaphylactic shock in bursectomized chickens. *Nature*, London. 205: 612-613.

*Scala, G., Corona, M., Delagalli, G.V. and Garmon, G. (1988). Involution of the bursa of Fabricius in the duck. *Anatomia Histologia Embryologia* **17**(2): 97-106.

Singh, U.B. and Sulochana, S. (1996). *Handbook of Histological and Histochemical Techniques*. Premier Publishing House, Hyderabad. 2nd Ed.

Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*. Oxford and IBH Publishing Co., New Delhi, 6th Ed. pp. 303-312.

Sugimura, M., Hashimoto, Y. and Yamada, J. (1975). Morphology of bursa of Fabricius in bursectomized and testosterone-treated ducks. *Jap. J. Vet. Res.*, **23**: 17-24.

*Thandavamoorthy, C. (1989) Microanatomical studies on the bursa of Fabricius in White Leghorns birds. Ph.D. Thesis, to Andhra Pradesh Agri. Uni., Hyderabad.

Thorbecke, G.J. Gardon, H.A., Wostman, B.S. Wagner, M. and Reynier, J.A. (1957). Lymphoid tissue and serum gamma globulin in young germfree chickens. *J. inf. Dis.* **101** : 237-251.

Tizard, I.R. (1996). The organs of the immune system: *Veterinary Immunology An Introduction*. W.B. Saunders Company, Philadelphia. 5th Ed. pp. 75-80.

Van Rautenfeld, Bernens, D. and Burdas, K.D. (1982). The bursa cloacae (Fabricii) of Struthioniformes in comparison with the bursa of other birds. *J. Morphol.* **172**: 123-129.

Ward, J.G. and Middleton, A.L.P. (1971) - Weight and histological studies of growth and regression in the bursa of Fabricius in the Mallard, *Anas platyrhynchos*. *Can. J. Zoo* **49** : 11-14

Warner, N.L. and Szenberg, A. (1962). Effect of neonatal thymectomy on the immune response of the chicken. *Nature*, London **196**: 784-785

Wolfe, H.R., Sheridan, S.A.; Wilstad, M.N. and Johnson, M.A. (1962). The growth of Lymphoidal organs and the testes of chickens. *Anat. Rec.* **142**: 483-493

*Yamada, J. (1966). The Weight and the histological changes with age of the bursa of Fabricius in chickens *Jap. J. Vet. Res.* **14**: 136

*Yamada, J., Sugimura, M. and Kudo, N. (1973). The weight and histological changes with age of the bursa of Fabricius in chickens. *Res. Bull. Obihiro. Univ.* **8**: 21-44.

*Yamada, J., Yamachita, T. and Mishu, M. (1977). Morphological studies of the bursa of Fabricius in Japanese quail. III. Histological changes with age. *Res. Bull. Obihiro. Univ.* **10**: 383-400.

* Originals not seen

POSTNATAL DEVELOPMENT OF THE BURSA OF FABRICIUS IN DUCK

(Anas platyrhynchos)

By

INDU V. RAJ.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Anatomy
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA

1999

ABSTRACT

The structure and post natal development of the bursa of Fabricius in White Pekin ducks were investigated using 51 birds aged from day-old to 155-days. The growth, morphology and histology of the bursa were studied using three birds of each age group.

In the day-old ducklings, the bursa could be seen as a smooth, yellow, elongated blind sac-like structure with a tapering apex. By 155 days it was a cylindrical and much reduced pale structure. In all the birds, the bursa communicated with the proctodeum of cloaca by a short stalk.

The inner surface of bursa contained two large well-developed plicae on the ventral aspect and about five to eight smaller folds all round the circumference. The number of plicae increased upto 30 days of post natal life. After 80 days a decreasing trend was recorded in their number.

After hatch, the bursal growth rate was not in proportion to that of body. It showed a decreasing trend after attaining peak values at five days of age. Though the bursa weight varied with variation in the age and body weight of the bird, a greater percentage variation in its weight was accounted for by body weight. The weight, length, diameter and plical measurements of bursa attained maximum

average values at 58 days of age indicating that the bursa of ducks may be most functional at this age.

Histologically, the wall of the bursa was divided into three tunics in birds of all ages. The outermost, tunica serosa enveloped the entire organ and increased in thickness gradually. The middle, tunica muscularis consisted of an outer circular and inner longitudinal layer of smooth muscle fibres with blood vessels in between. The innermost, tunica mucosa consisted of pseudostratified lining epithelium and lamina propria filled with follicles.

The epithelium was distinguished into follicle associated epithelium and interfollicular epithelium. Each follicle consisted of a cortex and medulla separated by a layer of epithelial cells with distinct basement membrane in birds of all ages. Lymphoblast, lymphocytes and macrophages formed the cellular component of the follicle. The number, size and cellular details of lymphoid follicles attained their peak-values by about 58 to 65 days of post hatch period.

The interfollicular and subepithelial connective tissue was made up of collagen and reticular fibres with a few elastic fibres. The cellular component in it included plasma cells, eosinophils, mast cells, macrophages and fat cells.

Involutory changes in the bursa were recorded from 95-days post hatch characterised by degeneration of plical epithelium and follicular atrophy. Prominent microscopic features of involution were evident from 140 days of age.

The bursa showed follicular degeneration, fibrosis of subepithelial stroma, collapse of plicae, depletion of lymphocytes and fatty replacement of the organ.

The lining epithelial cells of bursal mucosa revealed positive reaction to Schiff's reagent and metachromasia in birds of all ages. Intense acid phosphatase and moderate alkaline phosphatase activity was noticed in the epithelial cells of the bursa, in all the birds. The maximum positive immunoperoxidase activity seen by about 35 to 58 days of age suggested that immunologically the bursa was at its peak functional activity at this age.