PATHOLOGY OF THE HARDERIAN GLAND IN CHICKEN AND DUCK

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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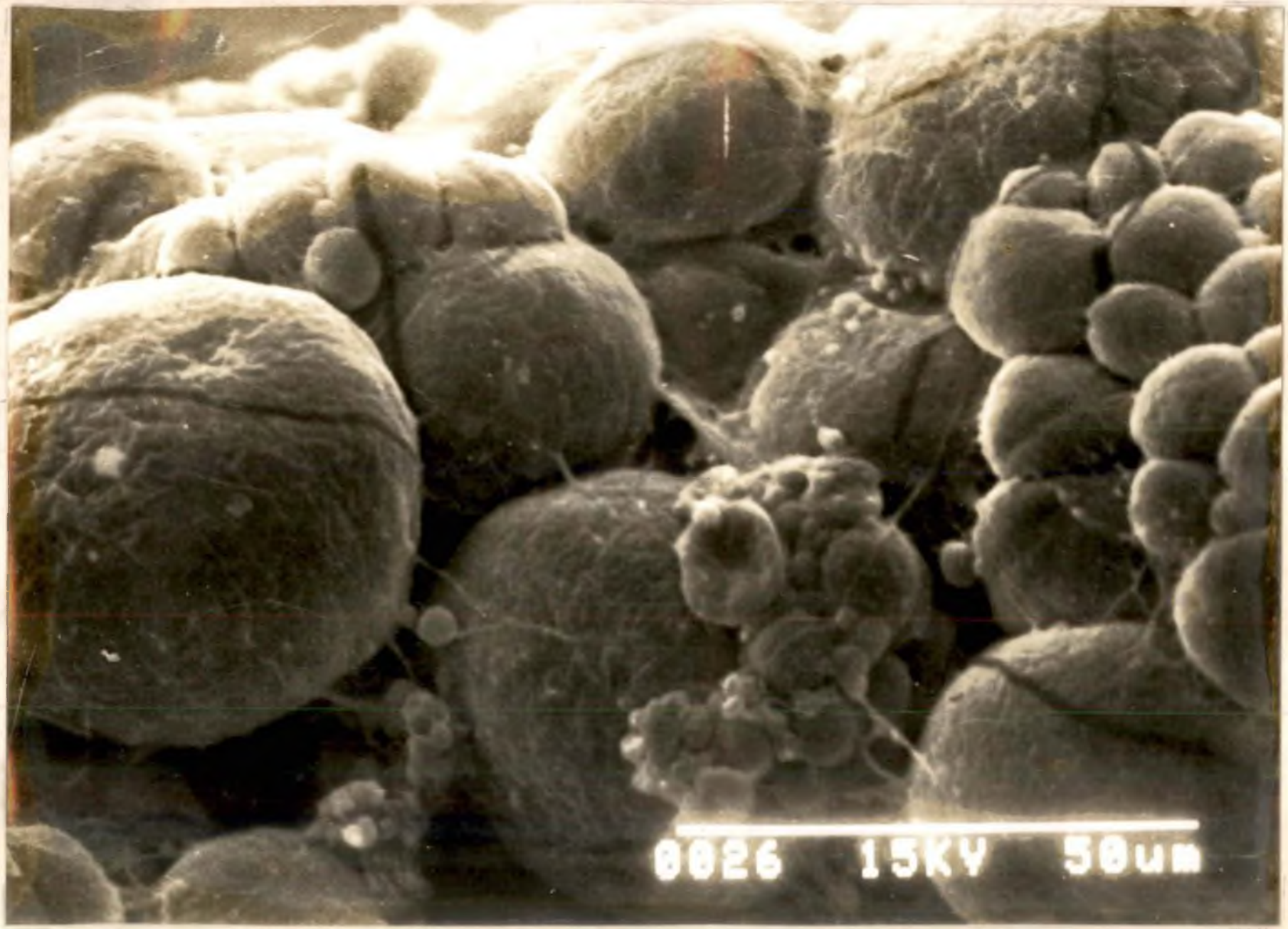
MANNUTHY, THRISSUR - 680651
KERALA

1998

LYMPHOID FOLLICLES

OF HARDERIAN GLAND OF TWELVE DAYS OLD CHICKEN

SCANNING EM VIEW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DECLARATION

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S. Mohan

Mannuthy,
27.02.1998.

S. MOHAN

CERTIFICATE

Certified that the thesis, entitled " PATHOLOGY OF THE HARDERIAN GLAND IN CHICKEN AND DUCK" is a record of research work done independently by Sri. S. Mohan, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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S. Mohan

Dedicated to my beloved mother
and in the memory of
my father

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LIST OF ABBREVIATIONS

Ach-E	-	Achtylcholine Esterase
AFC	-	Antibody Forming Cells
AGID	-	Agar Gel Immuno Diffusion
B	-	Bursa
BALT	-	Bronchial Associated Lymphoid Tissue
BSA	-	Bovine Serum Albumin
C	-	Control
CALT	-	Conjunctival Associated Lymphoid Tissue
CAMP	-	Cyclic Adenine Mono Phosphate
CGMP	-	Cyclic Guanine Mono Phosphate
D	-	Days
DP	-	Duck Plague
GALT	-	Gut Associated Lymphoid Tissue
g	-	Grams
h	-	Hours
HA	-	Haemagglutination
HALT	-	Head Associated Lymphoid Tissue
HI	-	Haemagglutination Inhibition
Ig	-	Immunoglobulin
IB	-	Infectious Bronchitis
IBD	-	Infectious Bursal Disease
IBV	-	Infectious Bronchitis virus
ILT	-	Infectious Laryngo trancheitis
IPC	-	Immunoglobulin Producing Cells
L	-	Left
mm	-	millimeter
ND	-	Newcastle Disease
NA	-	Neutralising Antibody
PAS	-	Periodic Acid Schiff
pH	-	Hydrogen ion concentration
R	-	Right
RD	-	Ranikhet Disease
SE	-	Standard Error
SPF	-	Specific Pathogen Free
SRBC	-	Sheep Red Biood cell
T	-	Thymus

1. INTRODUCTION

In Commercial poultry industry, flock health is a critical element and its success depends upon the health management. Intensive production methods, high density of bird population, management, nutritional and other genetic factors, may challenge the ability of a flock to resist infectious agents and remain disease free.

Chicken are provided with several mechanisms to protect themselves against pathogens. Some of these mechanisms are nonspecific defenses acting as simple barriers or scavenger systems such as mucous secretions. When infectious agents penetrate these barriers, the health of the bird depends upon its ability to recognise the invader and neutralize it. The result of the interaction between the defence system and the infectious agent will determine whether the bird will succumb to the disease or remain healthy. The result of the interaction has important economic implications.

Any plan for controlling a disease must be built around two main principles. First every effort must be made to prevent the infectious agent from reaching the birds. Secondly if it does, the birds must be able to resist the infections. Since the poultry environment has many infectious agents, the probabilities of one or more specific agents reaching the flock are high. Therefore, raising a disease free flock and maintaining a profitable flock depends largely on a strong immune system.

The immune response involves the dichotomus immune system. The chain of events which leads to the immune reponse can be simplified as, identification of the infectious agent as foreign to the body, analysis of its structural components or products; activation and changes in the immune cells producing a specific response and establishment of the immunological memory.

In the event of a second invasion the immune system "" remembers"" the agent and responds faster. The immune system will develop immune responses only against the specific disease to which the bird is exposed.

For years, the poultry industry has been taking advantage of these features of the immune system using vaccination programmes. Through vaccination, the immune system with the boosted effect resist specific pathogens before the disease manifest in the bird.

Birds have lymphatic aggregates widely distributed throughout the body, mainly in association with the respiratory and digestive systems and they are referred as secondary lymphoid tissues. This strategic placement of the immune cells is related to the most probable entrance path for infectious agents, which provides the system with the oppurtunity to respond promptly to the invasion of pathogens. The colonization of those lymphocyte patches begins with the hatching.

The secondary lymphoid tissues contributes significant role in the birds local immune reponse and the immune response is manifested in the form of secretions. The secretions are mostly of IgA rather than IgM or IgG. The major lymphoid tissues that are involved in the local immune response are the Head associated lymphoid tissues (HALT), Bronchial associated lymphoid tissue (BALT) and Gut associated lymphoid tissue (GALT).

Bronchus associated and gut associated lymphoid tissue (BALT and GALT) have both functional and morphological similarities and are involved in seeding lung, gut and other mucosal sites with predominantly IgA containing B cells. Both types of lymphoid tissues are engaged in the regulation and the controlled

amplification of immune responses, which vary from positive mucosal response in both mucosae and peripheral tissues to local mucosal responses and systemic tolerance.

The lymphoid tissue of the upper part of the respiratory tract includes paraocular and nasal lymphoid structures as well as some lymphoid accumulations in the pharynx and larynx. However, paraocular lymphoid tissue which is actually the Harderian gland (HG) produces tremendous number of plasma cells. The local antibody produced by the Harderian gland contributes in a major way to the local immune protection in the oculonasal and oropharynageal, upper respiratory tract region and thus have obvious relevance to the epidemiology and control of bacterial and viral diseases of poultry.

The Harderian gland has, therefore, significant role with the natural defence of the chicken. It is also significant to observe that during intraocular vaccination the antigens are processed by the Harderian gland and antibodies are produced which are highly immunologically reactive. Therefore in the present study it is proposed to investigate the structural changes in the gland following antigenic stimulation and to clarify the nature and extent of the differential responses in the chicken and the duck. The information obtained will help to modulate and manipulate the immune reponse during antigenic stimulation and a more effective and efficient vaccination response can be obtained.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 General

The presence of the Harderian gland was first reported in 1694 by Johann Jacob Harder in the deer. The Harderian gland has been described in all the terrestrial vertebrates, anurans, amphibia, reptiles, birds and mammals except the bats, terrestrial carnivores, cows, horses and higher primates.

Nebel (1696) was the first to work on the avian Harderian gland. MacLeod (1880) described first the histology and gross morphology of the duck Harderian gland.

Extensive studies were carried out on the avian Harderian gland starting from Ballantyne and Fourman (1967), Fourman and Ballantyne (1967) and Bang and Bang (1968).

2.2 Anatomy of the Avian Harderian gland

2.2.1 Gross Anatomy

Wight *et al.* (1971a) stated that the Harderian gland of the fowl was situated on the ventral and posterio-medial aspect of the eyeball. The Harderian gland is a flattened strap like (or) hour glass shaped structure in domestic fowl.

Wight and MacKenzie (1974) stated that the Harderian gland of the duck was situated on the posterio-medial aspect of the eye ball and it was almost hemispherical with a shallow concave face.

Burns and Maxwell (1979) stated that the Harderian gland of the duck emerged from the anterior extremity which opened into the medial aspect of the nictitating membrane in the domestic fowl.

Burns (1992) stated that the blood supply for the Harderian gland of the fowl was derived from ophthalmo temporal branch of the external opthalmic artery and nerve supply from the inferior branch of oculomotor nerve.

2.2.2 Histology of the Avian Harderian gland

Ballantyne and Fourman (1967) stated that the Harderian gland of the domestic duck was multilobular and consisted of many tubules lined with a single layer of columnar epithelium with basal nucleus.

Wight *et al.* (1971a) reported that the Harderian gland of the domestic quail, fowl and turkey was of compound tubulo-acinar type. The acini were located at the periphery of the tubules clustered around the secondary tubules to which tertiary collecting tubules were connected. The secondary tubules lead to single main collecting tubules. The acini and tubules were lined by columnar epithelium with spherical nucleus situated basally and the cytoplasm was more homogenous and eosinophilic. The epithelium of the tubules were almost cuboidal and large number of plasma cells accumulated beneath it.

Wight *et al* (1971b) reported that the Harderian gland of the fowl was surrounded by a thin connective tissue capsule and the septa divided the gland into lobules of varying size. From the capsule and septa, strands of collagenous and reticular fibres penetrate between the acini and tubules which also contained blood vessels and nerves. Foci of lymphocytes and autonomic ganglia were present in the capsule in addition to the fine elastic fibres.

Wight and Mackenzie (1974) stated that the single duct of the Harderian gland of the turkey, fowl and duck was lined by a single layer of epithelium. Lymphocytes either in diffuse form or with germinal centres were also found scattered along the length of the duct.

Survashe and Aitken (1978) reported that the draining duct of the avian Harderian gland consisted of low columnar to cuboidal epithelium interpersed with goblet cells and abundant lymphoid tissue.

Burns and Maxwell (1979) reported that the duct of the Harderian gland of the fowl and duck was lined by mucous secreting epithelium and of goblet cells.

Weaker (1981) reported that the acini with the Harderian gland of nine banded armadillo were drained by the intralobular ducts and the interlobular duct system of the proximal and distal portion of them appeared to form one excretory duct which emptied into the fornix of the conjunctiva associated with nictitating membrane.

Burns (1992) classified avian the Harderian gland into three types. (I) The compound tubulo acinar type I occur in fowl, dove , pigeon, (II) The compound tubular type II gland occur in pengiuns, pelicans, strakes, ducks and geese, and (III) The type III which is a mixture of type I and type II occurs in cranes, wadder and wood peckers.

2.2.3 Histochemistry

Ballantyne and Fourman (1967) reported that the central cells of the Harderian gland of the domestic duck contained many PAS positive granules. The central cells also contained metachromatic material positive for alcian blue. The cells in the Harderian gland of the domestic duck showed acid phosphatase activity but none showed alkaline phosphatase activity.

Wight *et al* (1971b) reported that the Harderian gland of the fowl was a mucous gland. The mucus was present in the acini and much of the tertiary tubules contained predominantly acidic sulphated mucosubstance.

Walcott *et al.* (1989) observed extensive acetylcholine esterase (AChE) network in the chicken Harderian gland.

2.2.4 Ultrastructure

Rothwell *et al.* (1972) in their ultrastructural studies of the Harderian gland of the fowl stated that the secreting columnar epithelium of the lumina consisted of four types of cells. Type I was of typical columnar epithelial cells with a circular or ovoid basally situated nucleus with an even chromatin pattern and a single nucleolus. Type II cells were characterized by the high degree of development of the golgi complex. Type III cell contained a streak of rough endoplasmic reticular lamellae in the basal part of the cell. Type IV cells were packed with secretory vesicles, the nucleus was angular and indented.

Maxwell *et al.* (1986) stated that the Harderian gland of the turkey contained characteristic bipolar epithelial cells and had abundant mitochondria, granular endoplasmic reticulum, ribosome and a complex network of golgi elements in the sub epithelial region.

2.2.5 Function of the Harderian gland

Burns (1976) stated that the main function of the Harderian gland in the domestic fowl was to lubricate the movement of the nictitating membrane, wet the surface of the eyeball, nourish the avascular cornea and that it played an important role in the local immunity of the eye and upper respiratory tract

Payne (1994) stated that the Harderian gland in terrestrial vertebrates, anurans, amphibia and reptiles had remarkably different features. The gland was a source of thermoregulatory lipids, pheromones and also acted as a photoprotective organ.

2.3 Immunocompetent cells of the Avian Harderian gland

Bang and Bang (1968) reported that the Harderian gland of the chicken was infiltrated with large population of plasma cells.

Wight *et al.* (1971a) reported the presence of few plasma cells and heterophils in the interstitium one day after hatching in the Harderian gland of the domestic fowl. According to them the cells appeared during the next six weeks.

Wight *et al.* (1973b) described that the plasma cells of chicken Harderian gland with bright eosinophilic cytoplasm and an eccentric nucleus which was usually small and pyknotic. The cells were circular in outline and were about 10.7 μm which was larger then most of the plasma cells (6.05 μm).

Burns and Mackenzie (1973) reported that the cholinesterase was found in the plasma cell and then that linked with fowl's immunological system.

Burns (1975) stated that the mott cells (Russell body containing plasma cells) occur in fowl's Harderian gland. According to him their number was followed the pattern of plasma cells number.

Kittner (1976) studied the lymphoid cells of the Harderian gland by light and Electron microscopy. He demonstrated Russell body containing plasma cells in the Harderian gland of the chicken.

Glick (1978) reported that the Harderian gland of the chicken contained heavily packed immunoglobulin surface determinant positive cells than that of bone marrow or caecal tonsils.

Survashe and Aitken (1978) reported that accumulation of heterophils and plasma cells soon after hatching and became more dense by four weeks in the Harderian gland of the fowl.

Schramm (1980) studied the Electronmicroscopic features of the Harderian gland of the chicken and reported the presence of Russell body in the plasma cells in the Harderian gland of the chicken.

Maxwell *et al.* (1986) found that the Harderian gland of the turkey contained myoepithelial cells and large number of plasma cells in the subepithelial region. They further stated that the number of plasma cells increased with the age.

Baba *et al.* (1988) stated that the lymphocytes of the Harderian gland were of bursa of Fabricius origin and were seeded into the Harderian gland prior to hatching and these did not appear to be involved in systemic immunity.

Gallego and Glick (1988) reported that the plasma cell had a high proliferation rate, approximately 2 to 3 times more than that of the spleen cells.

Del-cacho *et al.* (1991 and 1993) reported the presence of dendritic cell, myofibroblasts and fibronectin in the Harderian gland of chicken.

Fix and Arp (1991) reported that the Harderian gland of the chicken as a tubuloacinar secretory gland that contained a considerable plasma cell population. The infiltrating plasma cells in the Harderian gland were of bursal origin.

Mueller *et al.* (1991) described the modulation of the number of Russell bodies containing plasma cells in the Harderian gland under different experimentally induced immune conditions. The finding supported the concept of immunologic function of the Harderian gland which was similar to the function of the thymus on the one hand and bursa of Fabricius on the other hand.

Survashe (1992) stated that Russell body containing plasma cells of diverse morphology were a predominant feature of stimulated Harderian gland.

Glick (1994) stated that plasma cell of the chicken Harderian gland was recognised as one of the main organs capable of producing antibody producing cells of the body. The lymphoid cells of the fowl Harderian gland were derived from the bursa of Fabricius, thus implying that the gland might be active in humoral immunity.

Maslack and Reynolds (1995) reported the presence of CD3+, CD4+ and CD8+ T lymphocytes in the Harderian gland of the chicken.

Buzzell (1996) reported that the fowl's Harderian glands were probably lymphoid organs and were infiltrated with plasma cells which did not seem to be true of mammals.

Davinson *et al.* (1996) stated that the Harderian gland of the chicken consisted of 80% B lymphocytes and 20% T lymphocytes.

Scott and Savage (1996) reported that 6-9 weeks of age was the best time to find highly proliferative Harderian gland plasma cells and majority of the plasma cells were actively engaged in DNA synthesis.

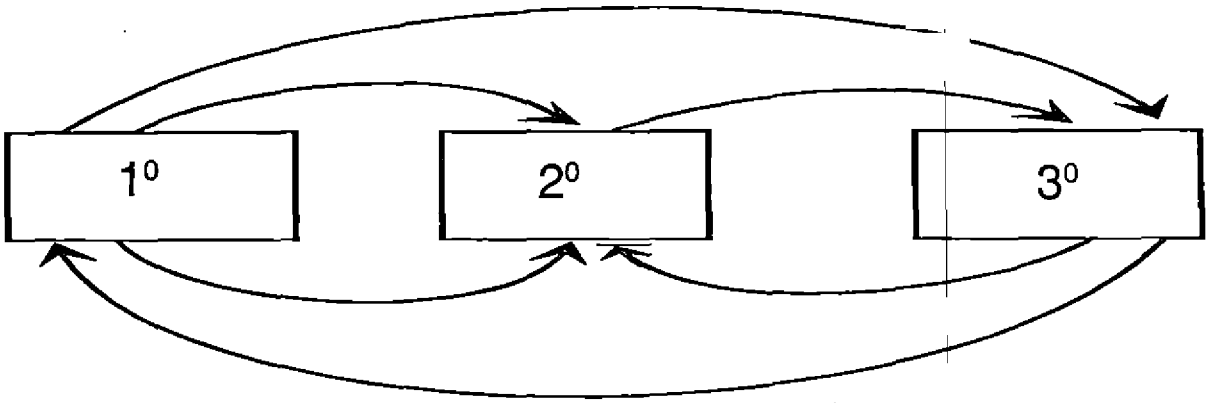
2.4 Immune response of the Chicken Harderian gland

2.4.1 Lymphoid Substance

Olah *et al.* (1996) classified that the surface epithelium of the central canal of the Harderian gland as a lymphoepithelial tissue which covered the dense lymphoid

substance. High endothelial venules were associated with the intense lymphocyte migration and this gave circumstantial evidence for a T - dependent region as found in a secondary lymphoid organ. The gland consisted of immunoglobulin A,M & G producing cells. These plasmocytic regions accounted for the immunosurveillance on the conjuctiva and in the upper respiratory tract through antibody production against bacterial or viral infection. By the influence of the local antigenic stimulus, the B - cells transformed into plasma cells which gradually appeared in the body of the gland. They gave the scheme for the placement of the Harderian gland among the lymphoid organs.

Lymphoid Organ



1⁰	2⁰	3⁰
Thymus	Spleen	Conjunctival associated lymphoid tissue (CALT) **Harderian gland (HG)** Gut associated lymphoid tissue (GALT)
Bursa of Fabricius	Lymph nodes	Bronchus associated lymphoid tissue (BALT)
Bone marrow	Tonsils	Skin associated lymphoid tissue (SALT)
Atigen independent and B cell differentiation Immunocomptent Virgin T and B cells.	antigen dependent immunologically Committed T and B cells	immunosurveillance tumor antigens, bacteria, Virus

2.4.2 Plasma cell proliferation

Savage *et al.* (1992) examined the percentages of proliferating plasma cells in the Harderian gland in chicks between 5 and 12 weeks of age. Two methods, 5 - bromo - 2' - deoxyuridine (Brd urd) incorporation into DNA and flow cytometric analysis of propidium Iodide (PI) stained cells, were employed in the control and emetine dihydrochloride treated birds. Flow cytometric analysis of PI stained cells in "S" phase were highest between 6 and 8 weeks of age. After this period of time, the number of "S" phase plasma cells decreased and remained low throguh 12 weeks of age. The lowest percentage of plasma cells in G0 and G1 phase were found at 6 and 8 weeks of age respectively and all ages had equal percentages of plasma cells in G2+M phase.

Scott and Savage (1996) described the proliferation of plasma cells in the chicken Harderian gland. At three and five days post treatment with emetine dichloride the plasma cell population decreased and by seven days of post treatment repopulation of the gland with plasma cell occurred. It was possible that the Harderian gland of chicken supported plasma cell proliferation through the elaboration of a factor which acted like a lymphokine.

2.4.3. Mitogenic response

Maslak and Reynolds (1995) adopted the blastogenic microassay to measure the blastogenic responses of lymphocytes from the chicken's Harderian gland. The T and B cell mitogen cultured lymphocytes obtained from the Harderian gland had highly significant mitogenic response to the T-cell mitogen and b-cell mitogen. This blastogenic response of the Harderian gland to mitogens may be indicative of its usefulness for measuring cell mediated responses.

Maslak and Reynolds (1995) identified B cells and sub-population of T-lymphocytes of the head associated lymphoid tissue of chicken using immunohistochemical staining. They reported that the concentration of T-lymphocytes, particularly CD3+, CD4+ and CD8+ in the Harderian gland of chicken increased with age but the concentration of B cell remained the same.

2.4.4. Response of the Dendritic cell, fibronectin and myofibroblast

Delcacho *et al.* (1991) reported that myoepithelial cells of the Harderian gland of the chicken got transformed into myofibroblasts under conditions of intense cell activity and were responsible for encapsulation of foreign bodies. They also suggested that the transformation might be a consequence of the functional hypoxia undergone by myoepithelial cells. The hypoxia could be due to the extended contraction which the cells were subjected to, in order to facilitate the excretion of the glandular secretion towards the conjuctiva.

Delcacho *et al* (1992) studied the relationship between the plasma cells, macrophages and the dendritic cells by means of ultrastructural localisation of the horse radish perioxide following local immunisation. After five days, perioxidase activity was found in macrophages and immature plasma cells. After nine days, peroxidase activity was found in dendritic cells. These results indicated that immature plasma cell in the Harderian gland could take up antigen.

Gallego *et al.* (1992) studied the follicular dendritic cell changes of chicken Harderian gland by Electron microscopy following administration of Salmonella-o-Antigen. They employed immunoperoxidase method for the detection of S-100 protein. They reported that the dentritic cells were closely associated with

lymphoblast and lymphocytes and S-100 protein was found only in dendritic cells. They suggested that during a secondary immune response, the follicular dentritic cell underwent a functional activation which involved morphological changes and phenotyphic expression of the S-100 protein.

Delcacho *et al.* (1993) stated that the myofibroblast might have a role in the synthesis and release of the intercellular electron dense material. In addition fibronectin was investigated by using immunoelectron microscopy as a component of this electron dense substance. They suggested that the fibronectin might bind both antigen and immune complexes, so that they can be more easily endocytosed by the plasma cell or attached to the dendritic cell surface during the immune response produced by the Harderian gland.

2.5 Immunoglobulins in the chicken Harderian gla

Bienenstock *et al.* (1973) studied the synthesis of IgG, IgA and IgM in chicken tissues. They reported that the Harderian gland is an exocrine gland of local immune response in the orbit and showed synthesis of IgA and IgM.

Albini *et al.* (1973 & 74) reported that upto fourth week after hatching, most of the plasma cells bore IgM as Immunoglobulin surface determinants (ISD), from the fourth to the nineth week, both IgG and IgA positive cells formed the bulk of the lymphoid cell population, whereas IgA alone was the predominant specificity of ISD.

Wick *et al.* (1974) characterised the immunoglobulin produced in the chicken Harderian gland. They reported the presence of IgG, IgM and IgA. According to them Harderian gland played the main role in the local immune mechanism of the upper respiratory tract.

Aitken *et al.* (1975) reported that the Harderian gland and lachrymal gland of chicken are the source of the lachrymal fluid. They secreted Immunoglobulin G, M, A. The secretion moreover has been found to express specific neutralizing antibody activity after experimental respiratory virus infection.

Davelaar and Kouwenhoven (1977) conducted an experiment with one-day old chicks which had maternal antibodies to infectious bronchitis virus (IBV) and they were immunised against infectious bronchitis with eye drops. The Harderian gland was shown by immunofluorescence to synthesize IgA after 2-3 weeks, and IgG after 2-4 weeks but not IgM.

Aitken and Survashe (1977) demonstrated large number of Immunoglobulin IgA producing cells in association with the upper respiratory tract in the Harderian gland of the chicken.

Glick *et al.* (1977) studied the immunoglobulin positive cells in the Harderian gland of the fowl. They reported the presence of IgA, IgG and IgM in the Harderian gland of chicken.

Ewert *et al.* (1979) stated that the highest density of plasma cells was in the Harderian gland of the chicken. IgG was the predominant class where as IgA and IgM plasma cells were present in almost equal but lower numbers. The Harderian gland plasma cells were the most likely source of salivary antibody.

Befus *et al.* (1980) stated that the Harderian gland was a paraocular mucosal associated lymphoid aggregate in the chicken and was enriched with IgA precursors. The Harderian gland, bronchus associated lymphoid tissue, conjunctival associated lymphoid tissue and Gut associated lymphoid tissue were a part of an extensive integrated mucosal associated lymphoid tissue system similar to mammals.

Halpern *et al.* (1981) examined the expression of retroviral antigen in the plasma cells of the chicken Harderian gland which individually produced IgM/IgA or IgG. They observed that the expression of endogenous retroviral envelop antigen of plasma cells of the chicken Harderian gland was at a higher level than the bursal cells.

Antonio zicca *et al.* (1982) studied the immunofluorescent patterns of cytoplasmic immunoglobulin (Clg) localisation in relation to the ultrastructure of maturing and degenerating B cells. It appeared that the Russell body formation was through the accumulation of Ig within the cisternae of the rough endoplasmic reticulum.

Burns (1982) demonstrated immunoglobulins, IgG gave the most intense fluorescence followed by IgA and IgM in GALT. The Harderian gland gave the most intense fluorescence of IgA followed by IgG and IgM.

Davelaar *et al.* (1982) reported the synthesis of IgA in the Harderian gland in chicks of 2, 3 and 4 weeks old. Some IgG was also observed, but IgM was absent from the gland.

Friederichs and Neumann (1983) reported that IgG positive cells were the first seen in the chicken Harderian gland. In one week after hatching a percentage of 34.3 percent was present, rising to 47 percent at two weeks and thereafter remaining steady, whereas they were first found in the bursa of fabrici in the 17th day embryo.

Walcott and McLean (1985) reported that the Harderian gland of the pigeon consisted of large population of lymphoid cells that produced IgA which is a significant component of the tears.

Mansikka *et al.* (1989) measured the antibodies produced by the Harderian gland of chicken in the tears before and after antigenic stimulation. In unstimulated chicken high levels of total IgM, IgA was observed. Expression of Ig genes was studied by using lambda L and μH chain specific DNA probes. In unstimulated chicken the concentration of μH and L chain m RNA in the Harderian gland was 8 times higher than in the bursa of fabricius or the spleen.

Baba *et al.* (1990) reported the role of the Harderian gland in the production of immunoglobulin, especially IgA. Lachrymal immunoglobulin almost disappeared after surgical removal of the Harderian gland and immunoglobulin produced by the Harderian gland cells was detected in the saliva but not in the trachea. Immunoglobulin production occurred in the Harderian gland cell culture in vitro and it consisted mostly of IgA. The production of IgM and IgG was very low. These findings indicated that lacrimal IgA was produced locally in the Harderian gland and lacrimal IgG and IgM were mostly transported from the blood.

Gallego *et al.* (1992) studied the immunoglobulin classes synthesised by the Harderian gland after local immunisation. They suggested that most of the IgG was found in tears after local immunisation and remarkable increase of IgA was also noticed.

Montgomery and Maslin (1992) stated that the Harderian gland of chicken was an important source of tear antibodies and played an important role in chicken's local immune response.

Olah *et al.* (1992) stated that although IgM, IgG and IgA producing plasma cells were present in the Harderian gland, only IgM and IgA positive cells were capable of a distinct relationship with epithelial cells.

Toro *et al*. (1993) stated that the lachrymal fluid which consisted of IgA secreted from the Harderian gland and IgG transferred from the serum in chicken gives information about the local immune status and is also likely to be useful for monitoring systemic humoral responses.

Tsuji *et al.* (1993) reported the role of the Harderian gland on the differentiation and proliferation of immunoglobulin A bearing lymphocyte in chicken. The mechanism of accumulation of surface immunoglobulin (SIgA) bearing cells in chicken Harderian gland was examined. Almost no SIgA bearing cells were identified in the Harderian gland of 1.5 week old chicken. In 3.5 week old chicken, however, 46.4 percent of the Harderian gland lymphocytes were IgA- bearing cells.

Brink *et al.* (1994) stated the existence of a functional link between the nervous and immune systems of the chicken Harderian gland. They have shown that the plasma cells of the Harderian gland bind an antibody to muscarinic acetylcholine receptor and that carbachol, acetylcholine increase the secretion rate of IgG. This neurotransmitter dependent increase of immunoglobulin secretion required an influx of Ca^{2+}.

Brink *et al.* (1994) described the role of membrane channels of IgG secretion by plasma cells in the chicken Harderian and lachrymal gland.

Cameran *et al.* (1995) reported that the chicken Harderian gland contained an abundance of plasma cells in the interstitium of the gland that secreted IgG, IgM and IgA. In an *invitro* preparation of this gland, the cholinergic agonist carbachol caused a transcient increase in the secretion rate of IgG. They investigated the effects of the cyclic mononucleotides CAMP and CGMP on this secretalogue response.

They postulated that muscarinic receptor activation led to a calcium influx that in turn led to an increased secretion rate of IgM.

Russell (1995) stated that the immunoglobulin levels in nine week old chicken were 36 percent IgA, 12 percent IgM and 32 percent IgG. Very efficient Harderian gland production of IgA, IgG and IgM by the Harderian gland can however be induced in the one day old chick. The immunoglobulin was shown to block the virus production related to upper respiratory tract diseases.

2.6 Response of the Harderian gland of chicken to antigens

Bang *et al.* (1972) described the lymphocyte depression induced in chicken on diet deficient in vitamin A. They stated that there was severe drop in the plasma cell number in the chicken maintained on vitamin A deficient diet and subsequent administration of Newcastle disease virus.

Parry and Aitken (1973) studied the immunoglobulin A in the respiratory tract of the chicken following exposure to Newcastle disease virus. They observed lymphoid and plasma cell aggregations in the respiratory tract, notably in the Harderian gland, They synthesized IgA antibody and could demonstrate IgA in the plasma cell of the Harderian gland by fluorescent antibody technique.

Babkin *et al.* (1974) studied the aerosol immunization against infectious laryngotracheitis in chicken using an inactivated vaccine - Ukrainian ILT. They noted that plasma cell reaction at 3, 7, 10 and 22 days after immunization was more pronounced in the Harderian gland than in the spleen, thymus, bursa Fabrici or bone marrow.

Krasnikou *et al.* (1975) reported the changes in the Harderian gland of hens with Marek's disease virus. There was multiplication of plasmacytes and desquamation of the glandular tissue, with necrosis of desquamated cells.

Aitken *et al.* (1976) stated that following primary exposure by the ocular route to lentogenic Newcastle disease virus (NDV) the lachrymal fluid, saliva and tracheal washes of three-week old specific pathogen free chicken acquired specific virus-neutralizing activity which considerably exceeded transudation of circulating antibody. Ocular infection induced marked lymphoid and plasma-cell activity in the Harderian gland and this was a major source of specific antibody in the lachrymal fluid.

Burns (1976) immunized chicken with bovine serum albumin (BSA) and reported that the Harderian gland has an important role to play in the local immunologic mechanism of the eye and upper respiratory tract of the fowl.

Davelaar and Kouwenhoven (1976) stated that conjunctival or intranasal infection of chicken 1 or 20 days old with infectious bronchitis (IB) vaccine virus resulted in great increase in vascularization of the stroma of the Harderian gland after three days. Between 7 and 21 days after vaccination there was pronounced formation of follicles which were composed of lymphocytes. Conjunctival and intranasal challenge with a virulent field virus of IB resulted in severe hyperaemia of the follicles and slight degeneration of the lymphocytes and plasma cells after 28 days.

Burns (1977) described the possible route of antigen uptake by the Harderian gland of fowl. He administered Indian ink and colloidal gold into the eye ball of chicken and pointed out that ocular administration was the best route by which exogenous antigens could reach the gland.

Davelaar and Kouvenhowen (1977) stated that the vaccination of day old chicks by conjunctival and intranasal routes with H120 infectious bronchitis vaccine resulted in obvious stimulation of the Harderian gland, and there was increased number of plasma cells and lymphocytes.

Parry and Aitken (1977) described the local immunity in the respiratory tract of the chicken, the secretory immune response to Newcastle disease virus and the role of IgA. Fluorescent localization of immunoglobulin producing cells (IPC) identified large number of plasma cells containing IgA in association with the upper respiratory tract, particularly in the Harderian gland which contained dense aggregation of plasma cells, many of which produced IgA.

Ewert *et al.* (1979) conducted an experiment in chicken inoculated with ND vaccine intraocularly and reported that highest density of the plasma cells was present in the Harderian gland. IgG was the predominant class, whereas IgA and IgM plasma cells were present in almost equal but lower numbers. The Harderian plasma cells were the most likely source of salivary antibody.

Pejkovski *et al.* (1979) described the immunosuppressive effect of infectious bursal disease (IBD) virus on vaccination against infectious bronchitis (IB). They reported delayed infiltration of the Harderian gland by lymphocytes and immunoglobulin cells.

Powell *et al* (1979) stated that after ocular administration to young chicken of sheep erythrocytes (SRBC), Newcastle disease virus (NDV), infectious bronchitis virus (IBV) homologous antibody was detected in the serum and in saline extracts of the Harderian glands. They suggested the paraocular glands are immunologically

responsive to topically applied antigens and that antibody can be detected in extracts of the stimulated Harderian glands.

Survashe *et al.* (1979) reported the local immunity produced in chicks by the Harderian gland and lachrymal gland after eye-drop application of live virus vaccine of Newcastle disease. They found heightened lymphoid activity and increase in the plasma cell numbers. However, the changes were more apparent in the lachrimal gland which normally carried few immunocompetent cells. After stimulation immunoglobulin containing secretion accumulated in the Harderian gland.

Davelaar and Kouwenhoven (1980) stated that protection by spray vaccination developed more slowly than by eye-drop application and this delayed protection coincided with a delayed lymphocytic infiltration and follicle formation in the Harderian gland as compared with eye-drop application.

Dohm's *et al.* (1981) recorded the plasma cell changes in the Harderian gland following infectious bursal disease (IBD) virus infection of the chicken. Plasma cell content of the Harderian gland was lowered among infected chicken from one to seven week post inoculation. Lymphoid follicles and heterophil population in the Harderian gland did not appear to be affected by IBD virus infection.

Avram and Bucor (1982) conducted an experiment in chicks that were vaccinated (aerosol) with lentogenic Newcastle disease virus. They stated that in vaccinated chicks plasmacytic cells and lymphoid mass were more apparent and they were located between the acini as well as around the excretory ducts of the Harderian glands. Russell's bodies and large PAS positive masses developed in the plasmacytes, coinciding with an increase in the Newcastle disease antibodies.

Davelaar *et al.* (1982) studied the synthesis and secretion of immunoglobulins by the Harderian gland of the fowl after eyedrop vaccination against infectious bronchitis (IBV) at one day old. The Harderian gland was shown by immunofuorescence to synthesize IgA after two to three weeks and IgG after two to four weeks but not IgM. IgM in tears increased from two to five weeks after immunization and the concentration in tears was higher than in serum. The results suggested mainly systemic production of IgG-IBV and an active and selective transport of IgG from the serum to tears.

Burns (1983) reported that fowls maintained on a zinc-deficient diet from hatching and immunized either by intraperitoneal injection of bovine serum albumin (BSA) or by a combination of ocular drops and intraperitoneal injections showed no antibody response as judged by immuno electrophoresis and immunofluorescence, whereas birds on a zinc sufficient diet had serum antibody and anti - BSA cells in the Harderian gland.

Ratanasethakul and Cumming (1983) conducted an experiment with 100 two-week old cockrels that were vaccinated with the A3 strain of IB Vaccine by conjunctival, intranasal, in-contact, drinking water and aerosol routes. Vaccination by the conjunctival and intranasal induced a good resistance to challenge, concurring with an obvious stimulation of the Harderian gland. The drinking water and aerosol route led to a low resistance to challenge, with minor change in the Harderian gland.

Sivanandan *et al.* (1986) described the histopathological changes induced by serotype II infectious bursal disease virus in specific-pathogen free chicken. They reported depletion of the plasma cells in the Harderian gland after ocular administration of IBD virus.

Lulticken *et al.* (1987) described the systemic and local antibody responses in chicken after infection and vaccination with infection bronchitis virus. The live IBV given by eyedrop stimulated the formation of not only IgA but also of IgG plasma cells in the Harderian gland. For the induction of a local (IgA) immune response in the Harderian gland of the chicken, the application of antigen should preferably be local. This local immunity induced after infection could not be boosted by parenteral immunisation with inactivated IBV.

Dohms *et al.* (1988) stated that plasma cells of the chicken Harderian gland were necrosed and there was damage of the lymphoid elements 5 to 14 days postinoculation of infectious bursal disease (IBD) virus. Other changes noted were haemorrhage and vacuolation of the glandular epithelium of Harderian gland.

Fix and Arp (1989) reported that after ocular administration the antigen was received by the conjunctival associated lymphoid tissue and through the duct it reached the Harderian gland, stimulated it and produced secretory IgA.

Mansikka *et al.* (1989) stated that after ocular administration of tetanus toxoid in chicken, specific antitetanus IgG and IgA antibodies appeared in the tears but IgM antibodies were barely detectable. The results indicated that after antigenic stimulation the Harderian gland B cells rapidly matured through IgM secretion to the production of IgG (or) IgA.

Butchner *et al.* (1991) studied the microscopic changes in the Harderian gland of specific pathogen free (SPF) chicken vaccinated and challenged with B15 and H13 strains of infectious bronchitis virus (IBV). Twenty-eight days following eyedrop

inoculation of seven day old SPF chicken with these vaccine viruses a marked increase in plasma cell numbers and in the formation of lymph nodules occurred in the Harderian gland. Lesions in the Harderian glands of unvaccinated chicken inoculated with H13-IB virus and B-15 viruses at 28 days of age by the eyedrop route included intense hyperemia, marked degenerative changes in the plasma cells and vesiculation of glandular epithelial cells.

Fix and Lawrence (1991) stated that in chicken conjunctival delivery of antigen produced an increase in Harderian gland plasma cells and specific antibody in ocular secretions.

Fix and Arp (1991) suggested antigen uptake by conjunctival route was the effective route. The antigen was received by conjunctival assosicated lymphoid tissue (CALT) and through the duct it reached the Harderian gland.

Montgomery *et al.* (1991) described the effects of Arkanas strain of infectious bronchitis vaccine on the head-associated lymphoid tissue of chicken. They observed increased plasma-cell counts and follicle number in the Harderian gland and lachrimal gland.

Gallego *et al.* (1992) compared ocular routes of antigen administration with eyedrop, ocular conjuctiva and injection into the nictitating membrane. Antigen was observed in the cytoplasm of macrophages after injection into the nictitating membrane. Number of germinal centres in the gland after injection into the membrane was higher than following the other two ocular applications. Sheep red blood cells were administered using these three routes leading to significantly more plaque forming cells in the Harderian glands of chicken, injected by the nictitating

membrane. It was suggested that injection into the nictitating membrane was the most effective ocular route for producing a local immune response in the Harderian gland.

Russell (1992) demonstrated that lachrimal IgG and IgM antibody occurred at one to nine percent of the titers of serum antibody to Newcastle disease virus (NDV) immunisation with inactivated virus or the passive transfer of NDV immune serum between chicken. This percentage increased to 13.33 percent of serum titers after intra-ocular infection with NDV as if the replication of NDV in the Harderian gland stimulated lachrimal antibody of all classes.

Tortuero and Barrera (1992) studied the histological changes in the Harderian gland of chicks after experimental intraocular inoculation with avian infectious bronchitis virus. They noted petechial haemorrhage and there was severe lymphocyte and plasma cell depletion.

Lockaby *et al.* (1993) detected Newcastle disease virus (NDV) antigens in the Harderian gland by using an immunoperoxidase histochemical technique in the specific pathogen free (SPF) chicken at 2, 5, 7, 10 and 14 days postinocuiation with Newcastle disease virus.

Russell and Coudert (1993) studied the regional antibody forming cell responses following administration of Newcastle virus. They reported that when the virus replicated in the conjuctiva and the Harderian gland it stimulated the production of IgA, IgG and IgM in the lachrimal fluid and the Harderian gland alone was responsible for lacrimal IgA production.

Russell and Koch (1993) reported that the Hitchner B1, and ulster strain of the Newcastle disease virus (NDV) replicated to high titer in the Harderian gland after eye-drop infection. The Harderian gland was the major site of antiviral IgA antibody forming cells (AFC) in the body and their number was correlated to the level of antiviral IgA antibody in the tears. Vaccines of the Hitchner B1 strain of NDV were much less effective in inducing antibody by the intranasal route compared with intraocular route and no virus was re-isolated after intranasal vaccination. The intravenous inoculation of inactivated Iscoms of NDV could stimulate the spleen, but not the Harderian gland to the same extent as a live virus.

Animas *et al.* (1994) conducted an experiment with two and six week old chicks that were inoculated with the Kagoshima - 34 strain of avian infectious bronchitis virus and the antibody content of Harderian gland, bile and serum was determined using neutralisation tests. The neutralising antibody (NA) in the serum, Harderian gland and the bile was detected earlier and in slightly higher concentration in the 6 - week - old chicken.

Montgomery *et al.* (1994) conducted an experiment with specific pathogen free (SPF) Leghorn chicks that were inoculated with different modified live infectious bronchitis virus (IBV) vaccines to determine if the vaccines interfered with immune complexes of the head region. The main histological changes associated with the vaccines were increase in the lymphocyte populations in the Harderian gland, lachrimal gland and nasal mucosae.

Russell (1994) inoculated ulster 2C and Hitchner B1, strain of Newcastle disease virus into inbred White Leghorn birds of the Reaseheath-C and 151 lines by the oculonasal routes. Both viruses replicated in the Harderian gland and induced virus

specific IgA in the tears and bile. The level of local virus replication in the Harderian gland positively predicted the local antibody response.

Toro and Fernandez (1994) assessed the relationship between the presence of Ig level secreted from the Harderian gland of chicken and the resistance showed by the birds against infectious bronchitis virus (IBV) challenge. Their results demonstrated that lachrimal fluid IgA levels in chicken was associated with resistance against IBV infection.

Jayawardane and Spradbrow (1995) studied the mucosal immunity in chicken vaccinated with V4 strain of Newcastle disease virus. Chicken vaccinated orally with the V4 strain of Newcastle disease virus and possessing low levels (or) undetectable levels of serum haemagglutination inhibition antibodies against Newcastle disease virus, resisted challenge with virulent virus. Lymphoid accumulations were detected in the trachea of chicken after vaccination and there was significant increase in the number of plasma cells in the Harderian gland of chicken after vaccination.

Russell and Ezeifeka (1995) stated that the Reaseheath line 'C' chicks produced IgA, IgG and IgM in their serum, tears, spleen and Harderian gland as a consequence of oculotopical vaccination with Hitchner B1 strain of Newcastle disease virus. The IgM response was seen first, at five days after vaccination and antiviral IgM levels in the tears and serum were negatively correlated to the level of virus in the Harderian gland over four to ten days post infection. They suggested that day-old chick responded well to the live virus vaccination and their IgM response was likely to have a role in the clearance of the virus.

Raj and Jones (1996) conducted an experiment in day-old SPF chicken and six week old broilers by inoculating intraocularly and intranasally with economically important variant of infectious bronchitis virus. They reported maximum isolation of the virus from the Harderian gland and bursa of Fabricious.

Toro *et al.* (1996) examined the histological changes in the Harderian gland induced by the attenuated H-120 infectious bronchitis virus (IBV) vaccine strain and the persistence of this virus in the stroma of the gland in chicken after eye drop vaccination. IBV vaccination with the attenuated vaccine strain H-120 resulted in partial damage to the Harderian gland. There was presence of both plasma cells showing Russell bodies and tubule epithelial cells exfoliation that occurred simultaneously with the presence of detectable IBV.

2.7 Effect of removal of the Harderian gland in chicken

Aitken *et al.* (1976) stated that functional ablation of the Harderian gland of chicken can be effected by occlusion of its draining duct, and then provided a means for evaluation of its immunological significance.

Neumann (1976) reported that after the removal of the Harderian gland in chicken the lachrimal gland 'took over' its physiological and immunological functions.

Neumann and Kaleta (1977) determined the humoral immune reponse by Haemagglutination inhibition test (HI) after surgical removal of the Harderian gland (HD - ex) and in normal chicken (HD-K) following conjunctival application of the Newcastle disease virus (strain Hitchrer B1). The results of the experiment in which the HD-K chicken developed higher antibody titers could not be reproduced in the HD- ex chicken. In both experiments the control chicken showed in comparision to the HD-ex chicken a tendency to increase IgM production.

Survashe and Aitken (1977) reported that three weeks after surgical removal of the Harderian gland, the lachrimal glands of ten - week - old fowls were heavier and contained more immunocompetent cells than the glands of intact birds. When the adult birds deprived of both paraocular glands were given sheep erythrocytes or Newcastle disease virus by eye drop they devloped slightly higher than normal titers of serum antibody but failed to produce lachrimal antibody. They postulated that after surgical excision of the Harderian gland there was a diversion to the lachrimal gland of immunocompetent cells originally destined to home to the Harderian gland.

Survashe and Aitken (1977) described the effects produced due to removal of the lachrymal gland and ligation of the Harderian gland of the fowl. Total or partial cystic degeneration of the Harderian gland and loss of immonoglobulins from lachrymal fluid was evident in three of six adult birds after operation. Functional deletion of these paraocular glands is thus feasible and can be used for investigations of local immunity of the oculonasal region.

Burns (1979) reported that surgical removal of the Harderian gland of the domestic fowl resulted in increased secretory activity in the lachrimal gland, increase in goblet cell numbers, along the length of the lachrimal gland duct and plasma cell were numerous in the lacrimal glands of operated birds and they were capable of antibody reponse to both systemic and topical applications.

Montgomery and Maslin (1989) described the effect of Harderian adenectomy on the antibody response in chicken. Intact chick and those that had their Harderian glands removed (GHx) at one day of age were studied for their reponse to optically or intraperitonially applied antigens. Removal of the Harderian gland resulted in a consistent decrease in antibody level in the tears regardless of the route of exposure.

2.3. Response of Harderian gland of chicken to Mycoplasma

Bencina *et al.* (1989) demostrated serum plate agglutination and Haemagglutination inhibition tests and an indirect immunoperoxidase assay on serum, respiratory secretion, synovial fluid and Harderian gland extracts on three to eight week old chicken that were experimentally infected with *Mycoplasma gallisepticum*. They observed that there was local antibody production in the Harderian gland.

Karaca *et al.* (1989) described the role of the Harderian gland in inducing resistance against *Mycoplasma gallisepticum* infection. Harderian glands of one day old chicken were surgically removed. At one week old, these chikckens and controls from which these tissues were not removed, were vaccinated intranasally with a temperate sensitive mutant of *Mycoplasma gallisepticum*. Humoral and local immunity were measured by means of antibody in sera and tracheal washings, respectively. They reported that removal of the Harderian glands neither affected the production of antibody to *Mycoplasma gallisepticum* nor altered the effectiveness of temperate sensitive *Mycoplasma gallisepticum* infection.

Bencinal *et al.* (1991)conducted an experiment with eight one year-old commercial layer hens that had strong humoral antibody response to *Mycoplasma synoviae*. They demonstrated an antibody response to *Mycoplasma gallisepticum* and *Mycoplasma gallinarum* in the Harderian gland and respiratory secretions. They reported that the Harderian gland of chicken had great role in inducing immune response to the upper respiratory tract diseases.

2.9 Parasitic diseases of the Harderian gland of chicken

Danley(1973) reported that excysted *Philophthalmus magalurus* metacercariae placed in embryonic chick Harderian glands maintained *in vitro*, migrated into the glands and appeared to feed on the cells and secretions. Excysted metacercariae placed in the orbit of chicks initially migrated into the Harderian gland but as they grew it moved back through the duct to its vestibule beneath the nictitating membrane. Only philophthalmids (*Philophthalmus hegneri* and *Parorchis acanthus*) showed a tendency to enter the Harderian gland.

Danley (1974) studied the host-parasite relationship of *Philophthalmus megalurus*. It was found to parasitize the Harderian gland in the chicken and induced immunoglobulin IgG and IgM after 10 days of primary infection.

Lauer and Fried (1974) studied the localization of *Philophthalmus hegeneri* in the eye of the domestic chick. Artificially excysted metacercariae of *Philophthalmus hegeneri* were placed on the eyes of anaesthetized chicks and their position was determined at autopsy one-day to three week later. During the first week, about half of the flukes recovered were in the Harderian gland duct and the remainder in the nictitating membrane.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The experiment was conducted at the Department of Pathology, College of Veterinary and Animal Sciences, Mannuthy to study the changes in the Harderian gland of chicks and ducklings following antigenic stimulation.

3.1 Chicks and Ducklings

Ninety-six, day old male chicks were purchased from the AICRP, Mannuthy and ninety six, day old ducklings of either sex were purchased from a private agency, Mannuthy.

3.2 Antigens

Antigens used for this study were

3.2.1. The freeze dried Ranikhet disease vaccine (Live - Lasota strain) of one ampule containing 100 doses obtained from Ventribiologicals, reconstituted in 3ml of sterile normal saline was used as antigen.

3.2.2. The freeze dried Infectious bursal disease vaccine (Live - Intermeidate strain) of one ampule containing 200 doses obtained from Ventribiologicals reconstituted in 3ml of sterile normal saline was used as antigen.

3.2.3. The freeze dried Duck plague vaccine (Live) of one ampule containing 200 doses obtained from IVPM, Ranipet reconstituted in 3ml of sterile normal saline was used as antigen.

3.3 Experimental design

Ninety-six, day old male chicks and ducklings of either sex were randomly divided into four groups, each consisting of twenty-four birds.

Group Days of	No. of birds Chicken	Duckling	administered	Type of vaccine sacrifice 4	8	12
Tc	24	24	Control	6	6	6
T_2	24	24	NDV	6	6	6
T_3	24	24	IBD	6	6	6
T_4	24	24	DPV	6	6	6
Total	96	96		24	24	24

Group I (T_C) was maintained as the control, Group II (T_2) was given Newcastle disease vaccine, Group III (T_3) was given Infectious bursal disease vaccine and Group IV (T_4) was given Duck plague vaccine.

One drop of the vaccine was administered intraocularly into birds of Group II (T_2), III (T_3), IV (T_4) from each experimental group consisting of twenty-four birds, six birds were slaughtered at three intervals namely 4th day, 8th day and 12th day.

The same number of control birds was slaughtered at the same time interval along with the experimental groups.

3.4 Harvesting of the Harderian gland.

The birds were decapitated, enucleation of the eye ball without damage was done and the glands were removed from the orbits. Gross changes of the gland were noted.

3.4.1. Fixation

The glands were fixed in 10% neutral buffered formalin and Carnoy's fluid for studying the histological changes.

3.4.2. Weight of the Bird and the Harderian gland

The weight of the bird was recorded before sacrificing and the weight of the Harderian gland after slaughter was noted using sartorius balance.

3.4.3. Harderian gland dimensions

The length, breadth (anterior and posterior) and thickness of the glands after harvesting were recorded by the use of vernier calipers to find the structural changes in the control and antigen inoculated groups.

3.5 Serum collection

Blood collected from the chicks and ducklings was left in the test tube for one h at room temperature, then kept at 4°C for six h for contraction of the clot and the serum separated was collected in sterile vial and stored at -20°C until used.

3.6 Chicken RBC

The blood was collected from chicken in Alsever's solution and it was centrifuged at 1500 rpm for 10 mts and the supernatent was discarded. Then it was washed three times with sterile normal saline and the packed RBCs were resuspended to give a 0.5 percent suspension.

3.7 Antibody detection :

3.7.1 Agar gel diffusion test (AGDT) : It was performed as per the method described by the Hiral *et al.* 1972.

Agar gel (1.25 percent) was prepared by dissolving 1.25 g agarose (SRL) in 8% sodium chloride solution containing one drop of 0.5% phenol. The mixture was heated slowly to the boiling point. . When the ingredients were fully dissolved the mixture was allowed to cool to 50°C. About 5ml of the hot gel was poured over the glass slide precoated with one percent melted agar and allowed to solidify and kept at 4°C. One central well and six peripheral wells with a diameter of 5mm with 2mm

interspace were cut with a template. The central well was loaded with antigen (IBD). Known positive and negative sera were loaded. The slides were incubated in moist chamber at room temperature for 48 to 72 h. The slides were examined for the appearance of precipitation lines.

3.7.2. Haemagglutination test (HA)

Two fold dilution of the live virus vaccine (RD) was made in normal saline in perspex haemagglutination plates. An equal quantity of 0.5 percent washed chicken RBC were added to each dilution. Suitable RBC and saline controls were also incorporated. The plates were incubated at room temperature for 45 min. The highest dilution showing haemagglutination (HA) was taken as HA titer (Poultry biologicals, 1963).

3.7.3. Haemagglutination inhibition test (HI)

Beta method of HI test was employed after ascertaining the HA titer of virus. Four HA units of the virus was prepared in normal saline. Serial two fold dilution of the serum was prepared in normal saline. Each of these dilution was mixed with 0.2 ml of four HA units of the vaccine virus and incubated at room temperature for 30 min. After this 0.4 ml. of 0.5 percent of suspension of washed chicken RBC was added to each well and mixed simultaneously.Virus, RBC and serum control were also kept. Following incubation for 30 minutes the HI antibody titer was taken as the highest dilution of the sera in which there was complete inhibition of HA (Poultry biologicals , 1963).

3.8 Histological studies

The tissues were processed by routine paraffin embedding technique (Luna, 1968) and the sections were cut at 5 - 6μ thickness. All the tissue sections were

stained with Harri's Haematoxylin and eosin (Bancroft and Cook, 1984). Special staining using Alcian blue pH 2.5, Methyl green - Pyronine Y, and Toluidine blue as described by Sheehan and Hrapchak (1980) was done as and when required.

The diameter of the plasma cells and the plasma cells containing Russell bodies were measured by the micrometer. Plasma cell assessment on 4th, 8th and 12th day of control and antigen inoculated groups were done asper Survashe *et al.* (1979).

The plasma cells were counted by selecting six randomly distributed fields at a magnifictaion of 10x X 100 in H & E sections and the score for the plasma cells were given as +, ++, +++ and ++++.

3.9 Scanning Electron microscopy Study

The Harderian gland tissues were processed and scanned as described by Kessel and Shih (1974) to focus the surface morphology.

3.10 Statistical analysis

Statistical analyses were done wherever required according to the methods described by Steel and Torrie (1960).

4. RESULTS

4.1 Weight of the Harderian gland

The mean weight of the gland (g)of the control group of chicks on the 4th day was 0.0096 (± 0.0008), 8th day was 0.0157 (± 0.0005) and 12th day was 0.0195 (± 0.0008) in chicks.

The mean weight of the gland (g) of the control groups of ducklings on the 4th day, 8th day and 12th day were 0.0019 (±0.0005), 0.00295 (± 0.0008), 0.038 (± 0.0012) respectively.

The average percentage body weight of the Harderian gland in the chick was found to be 0.020 - 0.033 and that of the duckling was 0.031 - 0.039. The weight of the Harderian gland of the ducklings was found to be more than that of the chicks.

The mean body weight , Harderian gland weight and percentage body weight of the Harderian gland in the antigen inoculated groups on the 4th day, 8th day and 12th day are shown in the table 1 and 2 respectively.

Comparative data of percentage body weight of the Harderian gland between groups of chicks and ducklings are shown in the fig 1.

The observation revealed that there was significant increase in the weight of the Harderian gland in the antigen inoculated groups.

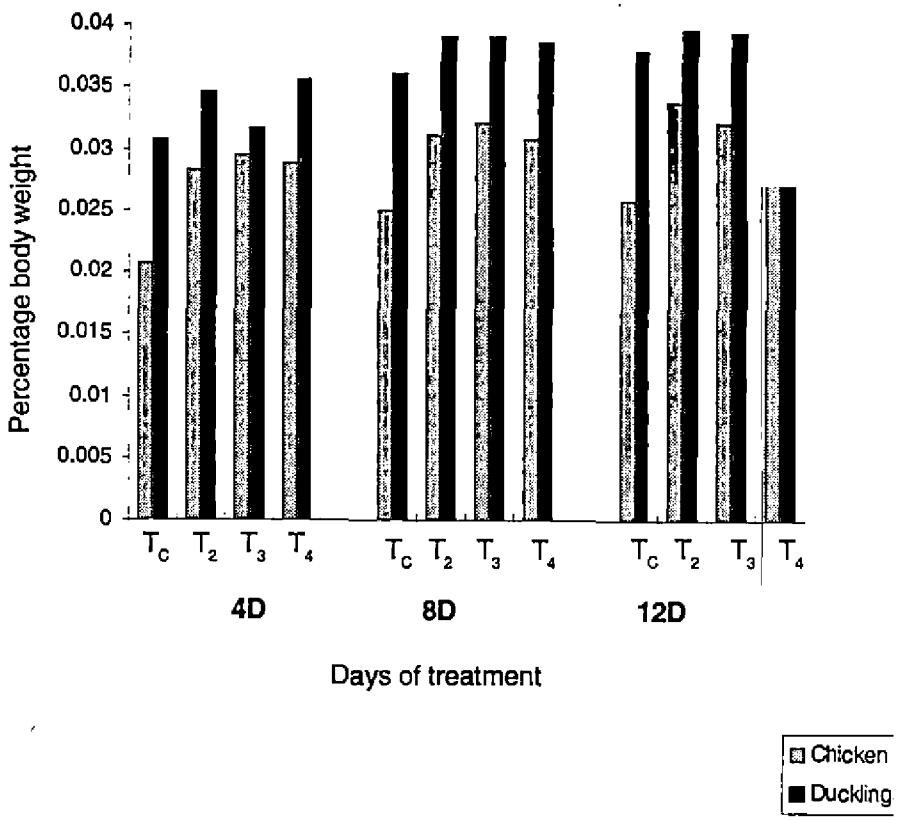
Table. 1. Mean body weight (g ± SE), Gland weight (g ± SE) and percentage body weight (± SE) of the Harderian gland in the control and antigen inoculated chicks

No.	Group	Body weight			Gland weight			Percentage body weight		
		4D	8D	12D	4D	8D	12D	4D	8D	12D
1.	T_c	44.33 + 1.74	62.33 + 182	75 + 2.67	0.0096 + 0.0008	0.0157 + 0.0005	0.0195 + 0.0008	0.0207 + 0.0012	0.025 + 0.0009	0.0258 + 0.0008
2.	T_2	45.00 + 1.34	60.67 + 1.98	80 + 2.48	0.0128 + 0.0006	0.019 + 0.0007	0.027 + 0.0012	0.0283 + 0.0008	0.031 + 0.0004	0.0338 + 0.0016
3.	T_3	43.67 + 1.20	64.0 + 1.35	82.33 + 0.61	0.013 + 0.0004	0.0207 + 0.0004	0.026 + 0.0005	0.0295 + 0.0008	0.032 + 0.0009	0.032 + 0.0006
4.	T_4	46.17 + 1.51	61.67 + 1.74	81 + 2.46	0.013 + 0.0005	0.0187 + 0.0004	0.0252 + 0.00008	0.0288 + 0.0009	0.0308 + 0.0007	0.031 + 0.0005

Table. 2. Mean body weight (g ± SE), Gland weight (g ± SE) and percentage body weight (± SE) of the Harderian gland in the control and antigen inoculated ducklings

No.	Group	Body weight			Gland weight			Percentage body weight		
		4D	8D	12D	4D	8D	12D	4D	8D	12D
1.	T_C	59.67 ± 1.41	80.33 ± 2.39	99.33 ± 3.08	0.019 ± 0.0005	0.0295 ± 0.0008	0.038 ± 0.00012	0.0307 ± 0.0013	0.036 ± 0.0009	0.0378 ± 0.0009
2.	T_2	60.67 ± 1.23	82.0 ± 1.71	98.67 ± 3.99	0.021 ± 0.0008	0.032 ± 0.0009	0.0388 ± 0.0009	0.0345 ± 0.0012	0.039 ± 0.0007	0.0395 ± 0.008
3.	T_3	62.0 ± 1.93	82.67 ± 1.76	104 ± 3.76	0.02 ± 0.0009	0.0323 ± 0.0008	0.0408 ± 0.00012	0.0315 ± 0.0009	0.039 ± 0.0006	0.0393 ± 0.0007
4.	T_4	61.17 ± 1.22	82.33 ± 1.58	104.83 ± 3.90	0.0218 ± 0.0008	0.0316 ± 0.0009	0.0415 ± 0.0002	0.0355 ± 0.0009	0.0385 ± 0.0007	0.0395 ± 0.0006

Fig. 1 Comparative data on the percentage body weight of the Harderian gland of the chicken and ducklings



4.2 Morphometry of the Harderian gland

The mean length (mm), breadth (mm)(anterior & posterior) and thickness (mm) of the control chick on the 4th day was 7.74, 2.23, 1.85 and 0.33; on the 8th day was 9.10, 2.93, 2.03 and 0.37 and on the 12th day was 9.16 , 2.96, 2.0 and 0.37 respectively.

The mean length (mm), breadth (anterior and posterior) (mm) and thickness of the control ducklings on the 4th day was 6.00, 3.12, 2.27 and 1.44; 8th day was 6.85, 3.36, 2.10 and 1.98; and the 12th day was 7.90, 4.00, 3.05 and 2.64 respectively.

The Mean diameter of the Harderian gland of the antigen inoculated chicks and ducklings on the 4th, 8th and 12th day are shown in the Table 3 & 4 respectively.

Comparative data of diameter of the Harderian gland (Length, Breadth & Thickness) on the 4th, 8th and 12th day are shown in the figures 2,3,4 and 5.

The observation revealed that there was significant increase in the geometrical measurements of the Harderian gland in the antigen inoculated groups.

4.3. Lesions in the Harderian gland of the chicks and ducks of the antigen inoculated groups.

4.3.1 Chicks

Mostly there was no gross appreciable changes. The hour glass shaped structure was retained in all the birds of the antigen inoculated chicks (Fig. 7, 9 & 11). On the

Table 3. Mean (mm ± SE) length, breadth and thickness of the Harderian gland in the control and antigen inoculated chicks

No.	Group	Length (mm)			Breadth (mm)						Thickness (mm)		
					Anterior			Posterior					
		4D	8D	12D	4D	8D	12D	4D	8D	12D	4D	8D	12D
1.	T_C	7.74 ± 0.118	9.10 ± 0.08	9.16 ± 0.14	2.23 ± 0.05	2.93 ± 0.03	2.96 ± 0.07	1.85 ± 0.03	2.03 ± 0.04	2.0 ± 0.02	0.33 ± 0.01	0.37 ± 0.007	0.37 ± 0.09
2.	T_2	8.96 ± 0.183	10.18 ± 0.09	10.03 ± 0.11	2.54 ± 0.05	3.30 ± 0.03	3.26 ± 0.06	2.05 ± 0.02	2.33 ± 0.02	2.29 ± 0.02	0.38 ± 0.009	0.44 ± 0.01	0.44 ± 0.01
3.	T_3	8.95 ± 0.18	10.04 ± 0.13	10.03 ± 0.14	2.76 ± 0.05	3.20 ± 0.08	3.18 ± 0.08	2.035 ± 0.03	2.42 ± 0.05	2.43 ± 0.06	0.41 ± 0.008	0.41 ± 0.02	0.44 ± 0.008
4.	T_4	8.69 ± 0.157	9.99 ± 0.14	10.06 ± 0.10	2.60 ± 0.06	3.22 ± 0.06	3.21 ± 0.007	2.02 ± 0.03	2.47 ± 0.05	2.32 ± 0.05	0.406 ± 0.01	0.43 ± 0.007	0.45 ± 0.01

Table 4. Mean (mm ± SE) length, breadth and thickness of the Harderian gland in the control and antigen inoculated ducklings

No.	Group	Length (mm)			Breadth (mm)						Thickness (mm)		
					Anterior			Posterior					
		4D	8D	12D	4D	8D	12D	4D	8D	12D	4D	8D	12D
1.	T_C	6.00 ± 0.1	6.85 ± 0.05	7.90 ± 0.08	3.12 ± 0.07	3.66 ± 0.04	4.00 ± 0.09	2.27 ± 0.06	2.103 ± 0.02	3.05 ± 0.08	1.44 ± 0.06	1.98 ± 0.06	2.64 ± 0.07
2.	T_2	6.32 ± 0.08	7.25 ± 0.05	8.55 ± 0.07	3.21 ± 0.06	3.60 ± 0.06	4.14 ± 0.03	2.29 ± 0.04	2.35 ± 0.05	3.19 ± 0.07	1.38 ± 0.09	2.06 ± 0.03	2.62 ± 0.10
3.	T_3	6.31 ± 0.07	7.20 ± 0.05	8.56 ± 0.08	3.22 ± 0.06	3.71 ± 0.03	4.00 ± 0.08	2.30 ± 0.02	2.30 ± 0.04	3.20 ± 0.04	1.42 ± 0.04	2.08 ± 0.09	2.66 ± 0.07
4.	T_4	6.36 ± 0.09	7.15 ± 0.06	8.54 ± 0.06	3.27 ± 0.06	3.68 ± 0.02	4.06 ± 0.07	2.28 ± 0.05	2.31 ± 0.03	3.20 ± 0.08	1.53 ± 0.04	2.05 ± 0.09	2.72 ± 0.07

Fig. 2 Comparative data on the Length of the Harderian gland. of the chicken and ducklings

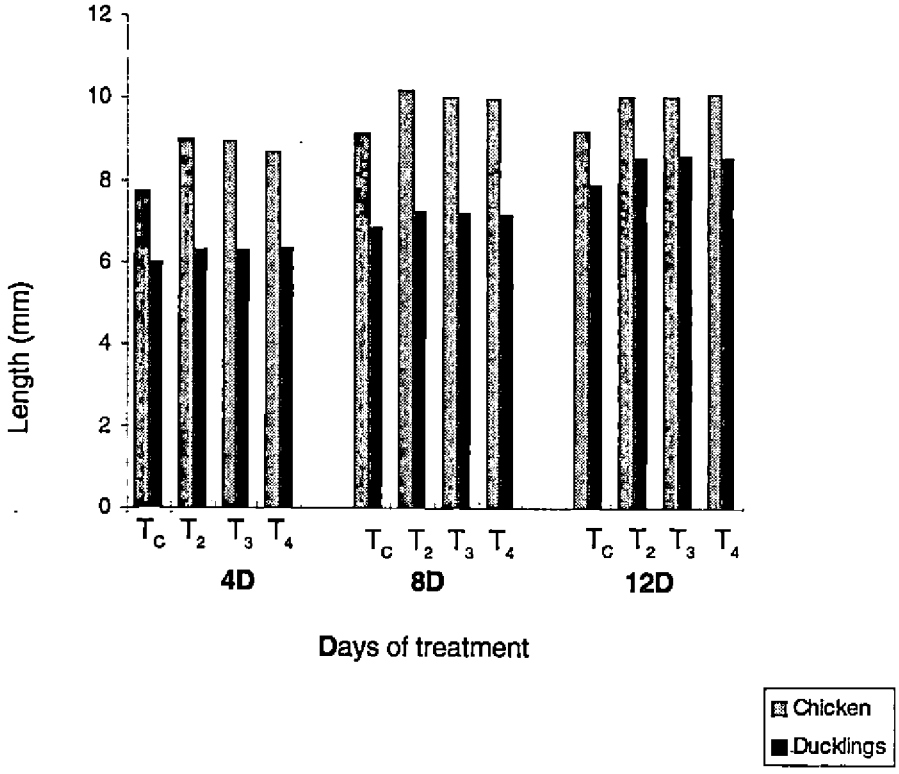


Fig. 3 Comparative data on the Breadth (anterior) of the Harderian gland of the chicken and ducklings

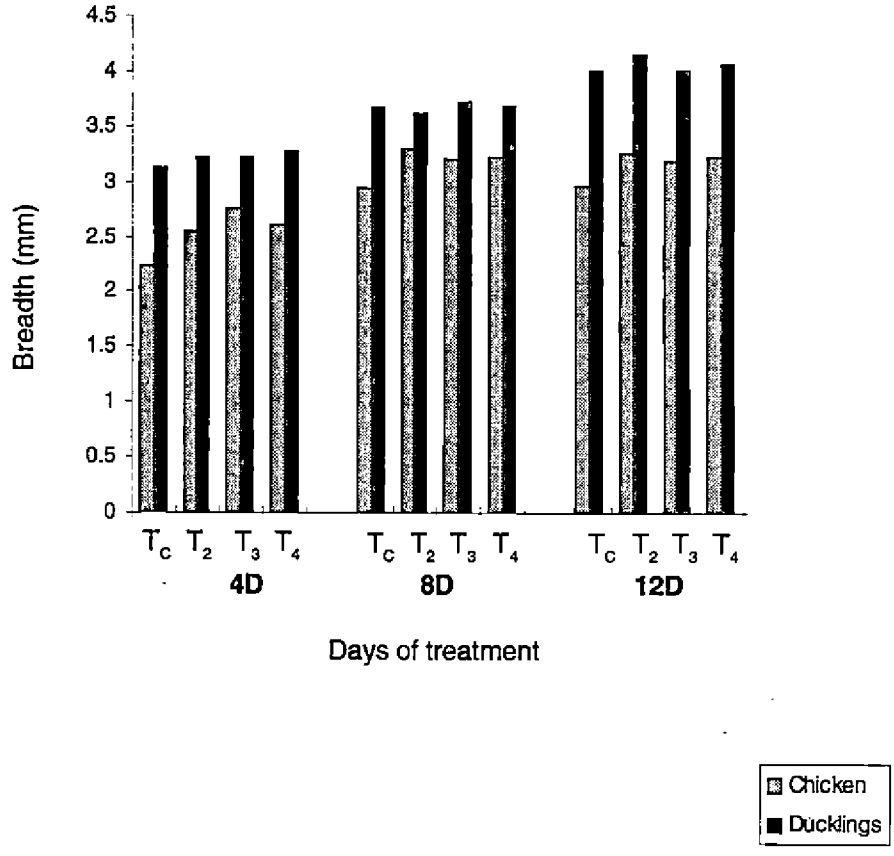


Fig. 4 Comparative data on the Breadth (posterior) of the Harderian gland of the chicken and ducklings

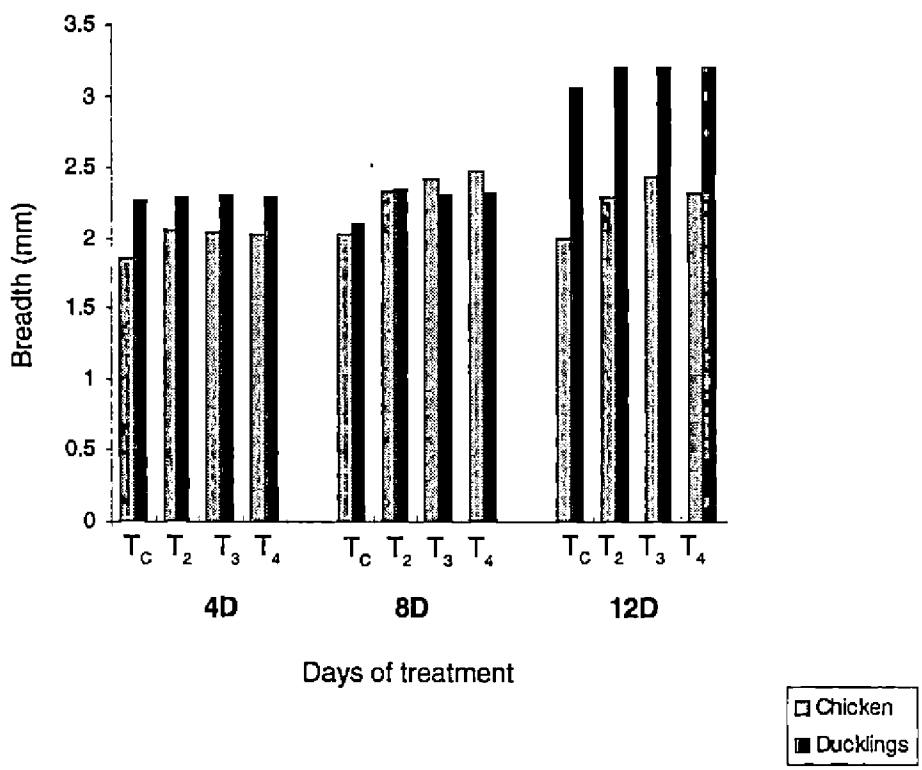
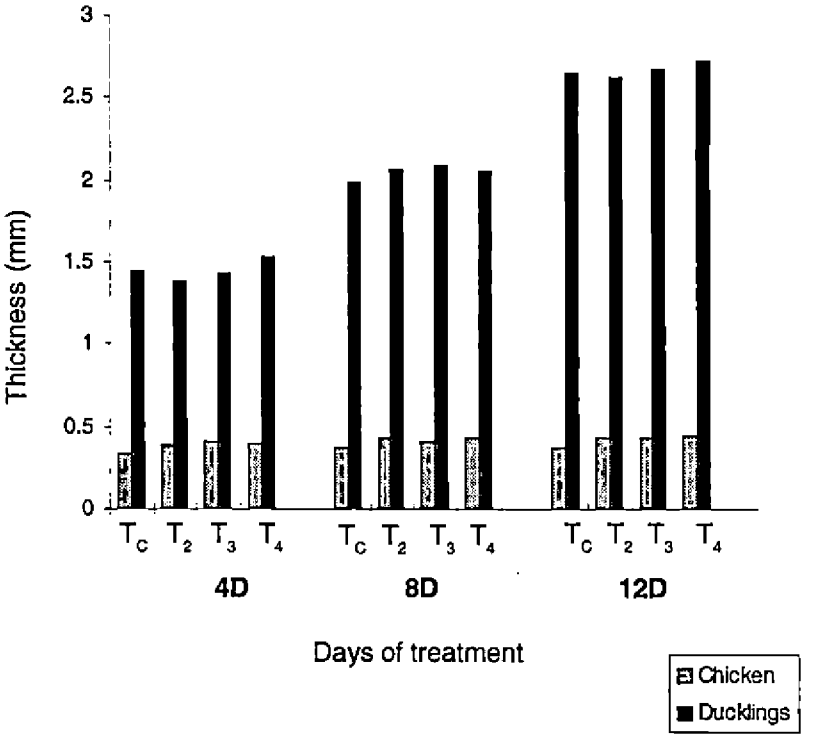


Fig. 5 Comparative data on the thickness of the Harderian gland of the chicken and ducklings



8th day one bird in the Group III exhibited mild congestion. On the 12th day two birds in the Group IV exhibited petechiae. Rupture of the capillaries on the 12th day in the Group III was observed.

4.3.2. Ducks

There was no gross appreciable changes. The club shaped structure was retained in all the birds (Fig. 8, 10, 12). On the 12th day some of the birds in the Group IV showed mild congestion.

4.4. Histological changes in the Harderian gland following antigenic stimulation

The histological changes in the Harderian gland of the chicks following antigenic stimulation by vaccine virus (RD, IBD and DP) showed the following histological changes.

Section of the Harderian gland of chicks on the 4th day showed congestion and mild degenerative changes of acinar epithelial cells (Fig. 13). The plasma cells infiltration was very less but more than that of the control. Heterophilia was evident (Fig. 13). Lymphoid foci were very less (Fig. 14 & 15).

Sections on the 8th day showed moderate heterophilia (Fig. 16 and 17).Congestion of the interlobular blood vessels and vacuolation of the epithelial cells of the acini and tubules were evident (Fig. 16). Lymphoid and plasma cells were more when compared to the 4th day of the antigen inoculated groups (Fig.18). Increased vascularity of the stroma was observed (Fig. 19). Russell bodies containing plasma cells containing Russell body were appreciated in the interstitial tissue (Fig.20).

Sections on the 12th day showed heightened lymphoid and plasma cell activities (Fig.21 and 22). Russell body contaning plasma cells were significantly increased in the interacinar tissue (Fig. 23). Appearance of macrophages were evident in large numbers. Exfoliation of the epithelial cells were seen (Fig. 24). There was increased thickening of the connective tissue fibres(Fig. 25). Some individual plasma cells displayed nuclear pyknosis. Mild infiltration of heterophils was evident (Fig.25).

4.5 Observations of plasma cells

The plasma cells had brightly eosinophilic cytoplasm and eccentric nucleus (Fig. 20, 23 and 26). They were spherical to global. The diameter of the plasma cell measured by the micrometer was 8.2 µm (S.E. 0.308 µm) and the Russell body containing plasma cells was 8.4 (S.E. 205 µm) in 1000X of the light microscope.

The Russell body containing plasma cells were slightly larger than the other plasma cells (Fig. 20 & 23).

The staining with Methylgreen pyronine showed the plasma cell nucleus light green (Fig. 27).

4.6. Plasma Cell Count

The plasma cell count of the Harderian gland of the chicks on the 4th, 8th and 12th day was evaluated.

It was found that the plasma cell count in the RD Vaccine virus inoculated groups was more when compared to the other two groups of IBD and DP Vaccine virus exposed. The DP Vaccine virus stimulated birds was found to have least plasma cell count than the other two Vaccine virus (RD and IBD) inoculated groups.

The plasma cell count of the chicks on the 4th, 8th and 12th day is showed in the Table 5.

Comparative data on plasma cell count of the Harderian gland between groups of chicks are shown in Fig 6.

4.7. Comparative histological changes in the Harderian gland of the ducklings and chicks following antigen inoculation.

Degenerative changes in the tubular epithelial cells of the duck were found on the 4th, 8th and 12th day (Fig. 28 and 29). On the 8th day dilatation of the capillaries and increased vascularity of the stroma was found (Fig. 30).

Vacuolation of the epithelial cells and mild thickening of the fibrous tissue was seen on the 8th day (Fig. 31). Increased thickening of the fibrous tissue and congestion of the blood vessels on the 12th day was evident (Fig. 32).

The plasma cell response was seen in the antigen inoculated group on the 12th day only. Very few plasma cells and lymphoid cells were sparsely scattered on the interlobular tissue on the 12th day (Fig. 34 & 35). There was no significant increase of plasma cells on the 12th day. Russell body containing plasma cells were not found in the tissue as such in chicks.

4.8. Histochemistry

Methylgreen pyronine positive cells were demonstrated in the chicks (Fig.27). Metachromasia was appreciable in the epithelia of both chicks and ducks (Fig. 38 & 39). The epithelia in the chicks and ducklings were Alcian blue pH(2.5) positive (Fig. 36 & 37).

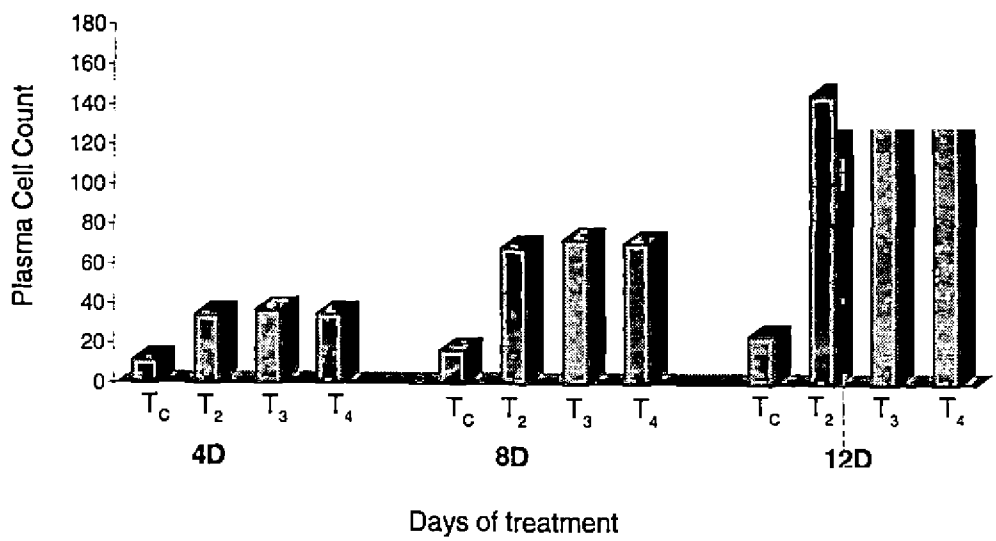
Table 5. Mean Plasma cell count (± SE) of the

Harderian gland in the control and antigen inoculated chicks

No.	Group	Plasma cells/6 fields x 1000			Score		
		4D	8D	12D	4D	8D	12D
1.	T_c	11.04 + 0.37	16.33 + 0.64	23.67 + 0.88	+	+	+
2.	T_2	33.92 + 1.53	68.21 + 1.67	144.88 + 3.49	++	+++	++++
3.	T_3	36.54 + 1.37	72.08 + 0.46	160.08 + 2.52	++	+++	++++
4.	T_4	34.33 + 1.18	70.46 + 2.27	147.67 + 3.74	++	+++	++++

\+ : Less
++ : Moderately high
+++ : High
++++ : Very High

Fig. 6 Data on the Plasma cell count in the Harderian gland of the Chicken

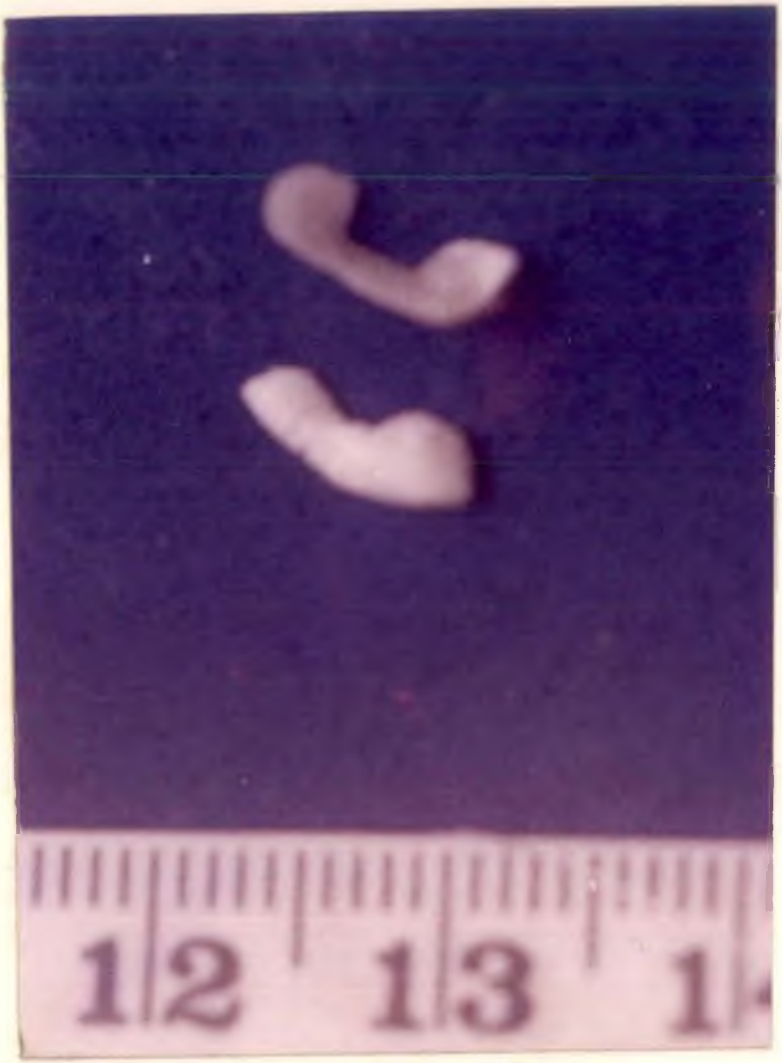


4.9. Antibody response

The freeze dried Ranikhet disease Vaccine (Live-Lasota) inoculated chicks and ducklings on the 12th day revealed an antibody titer of 8 by HI test, Infectious bursal disease Vaccine (Live - Intermediate) inoculated chicks and ducklings on the 12th day revealed faint precipitating in AGID. The serum sample from the control birds revealed no antibody response.

PHOTOGRAPHS

Fig. 7 Harderian gland of the four days old chicken, control (L) and the enlarged gland from the antigen inoculated (R)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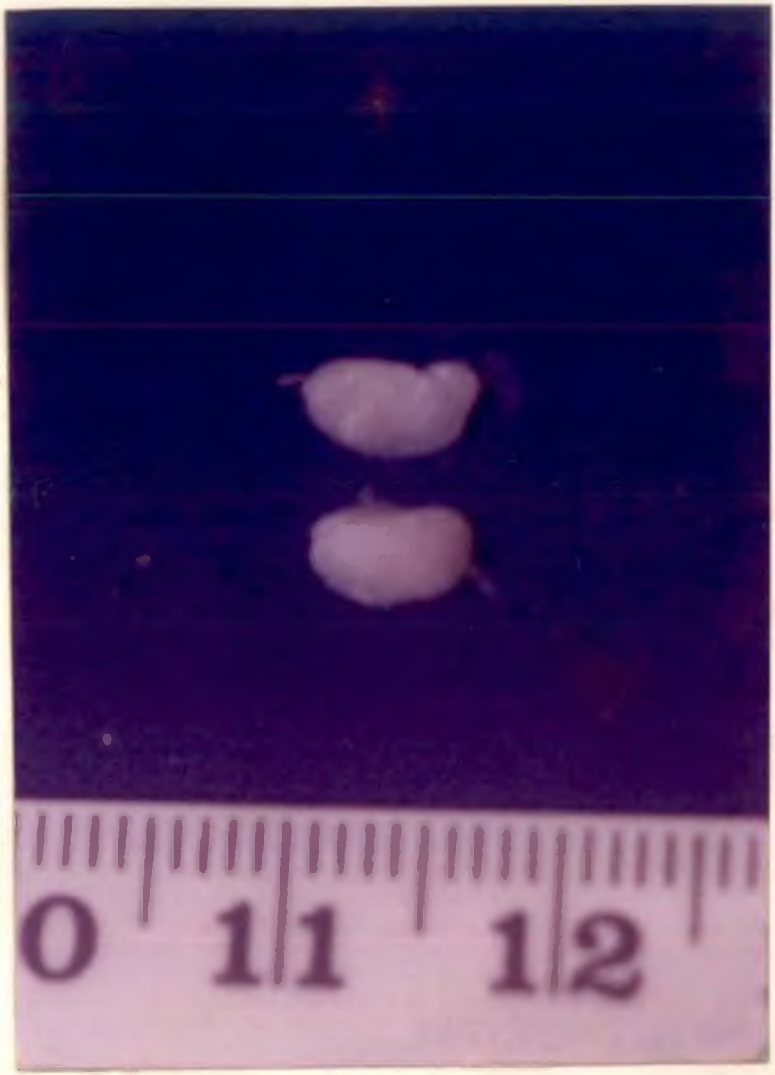
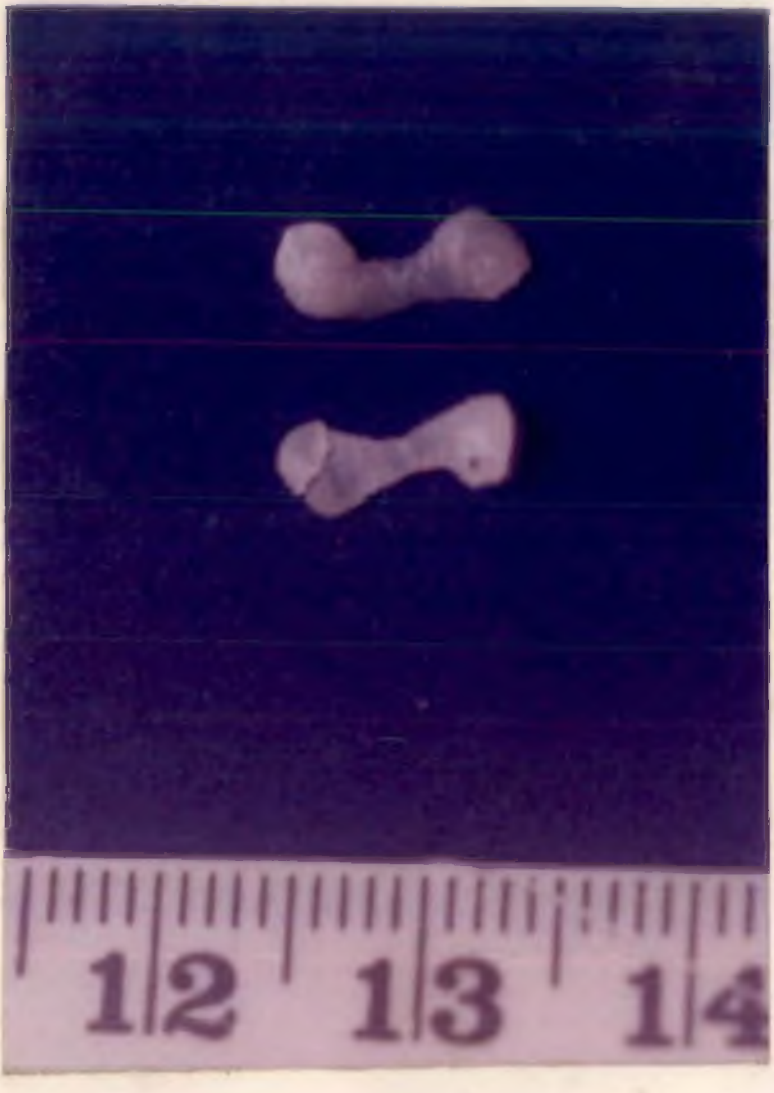

0
1 1/1
1 1/2

Fig. 9 Harderian gland of the eight days old chicken, control (L) and the enlarged gland from the antigen inoculated (R)

Fig. 10 Harderian gland of the eight days old duckling, control (L) and the enlarged gland from the antigen inoculated (R)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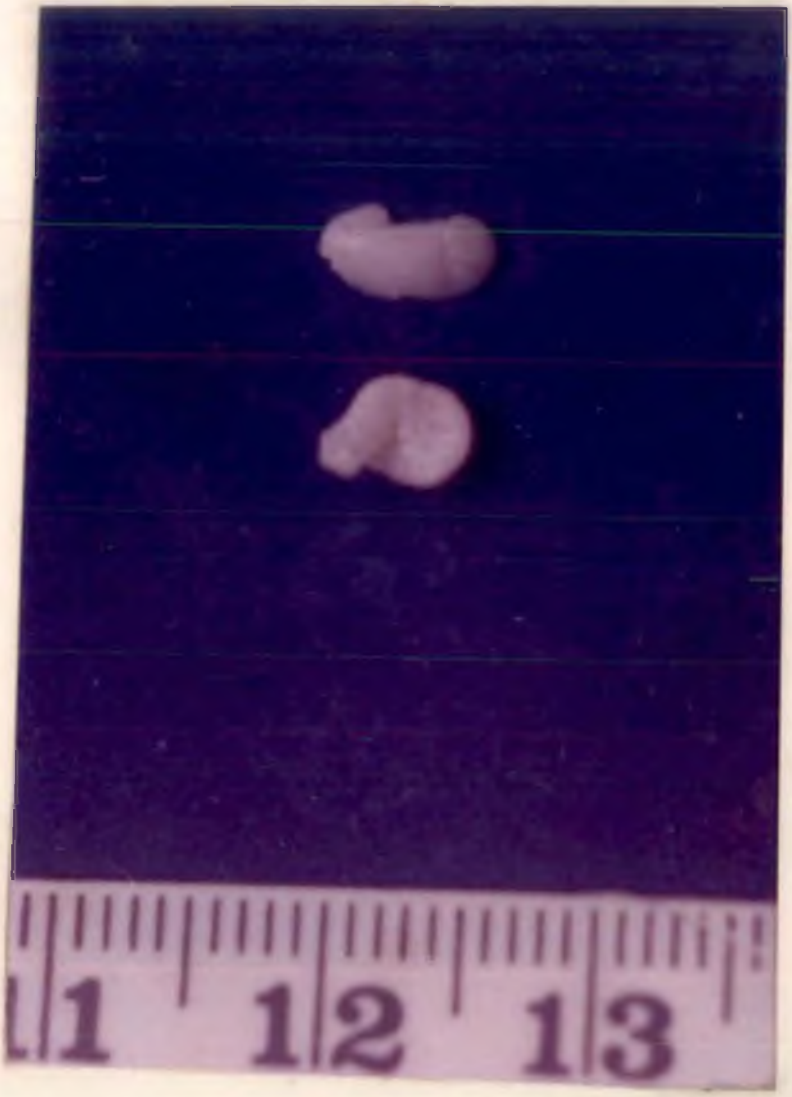
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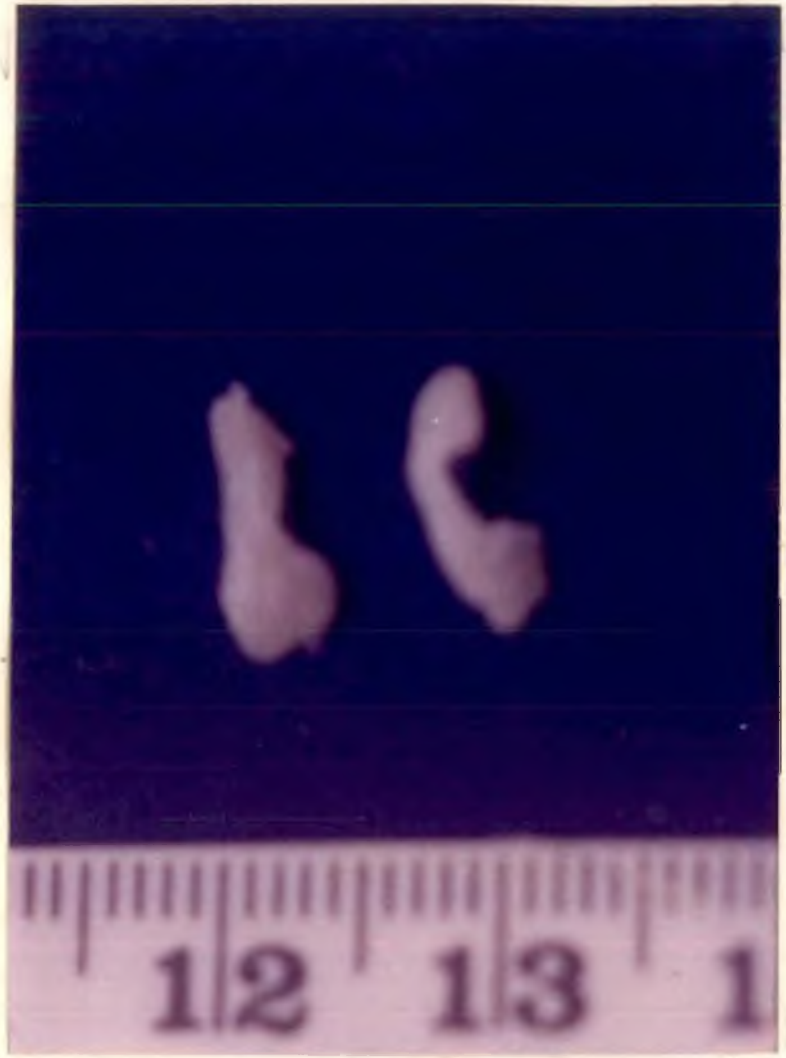
Fig. 11 Harderian gland of the twelve days old chicken, control (below) and the enlarged gland from the antigen inoculated (above)

Fig. 12 Harderian gland of the twelve days old duckling, control (L) and the enlarged gland from the antigen inoculated (R)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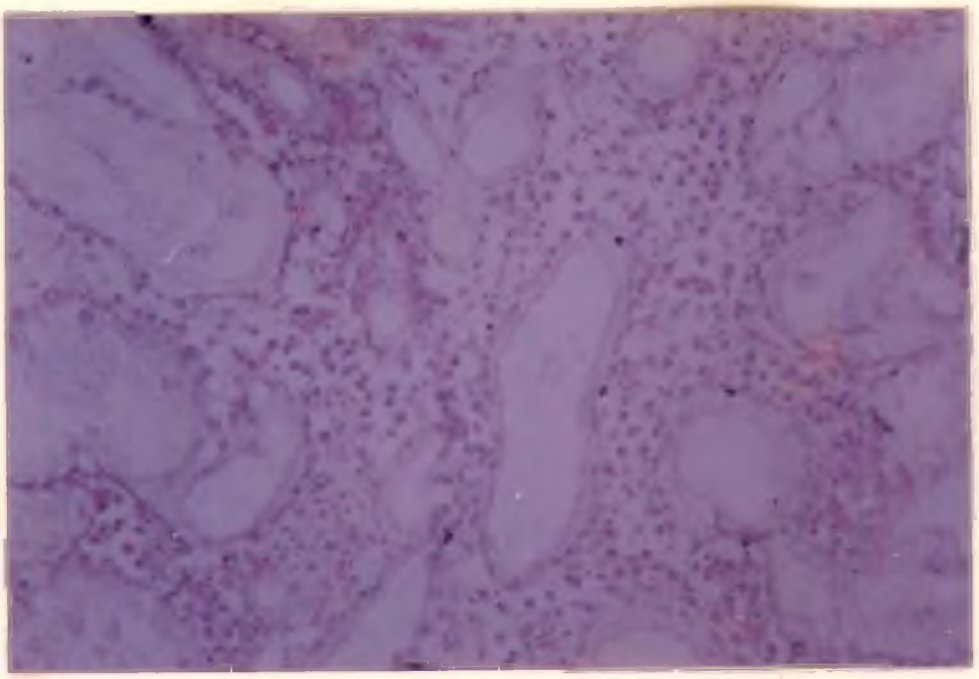
14

Fig. 13 Harderian gland of four days old chicken, four days after exposure to RD vaccine virus showing infiltration of plasma cells. H & E x 250.

Fig. 14 Harderian gland of the four days old chicken, four days after vaccination with RD vaccine virus showing lymphoid cell infiltration. H&E x 160.



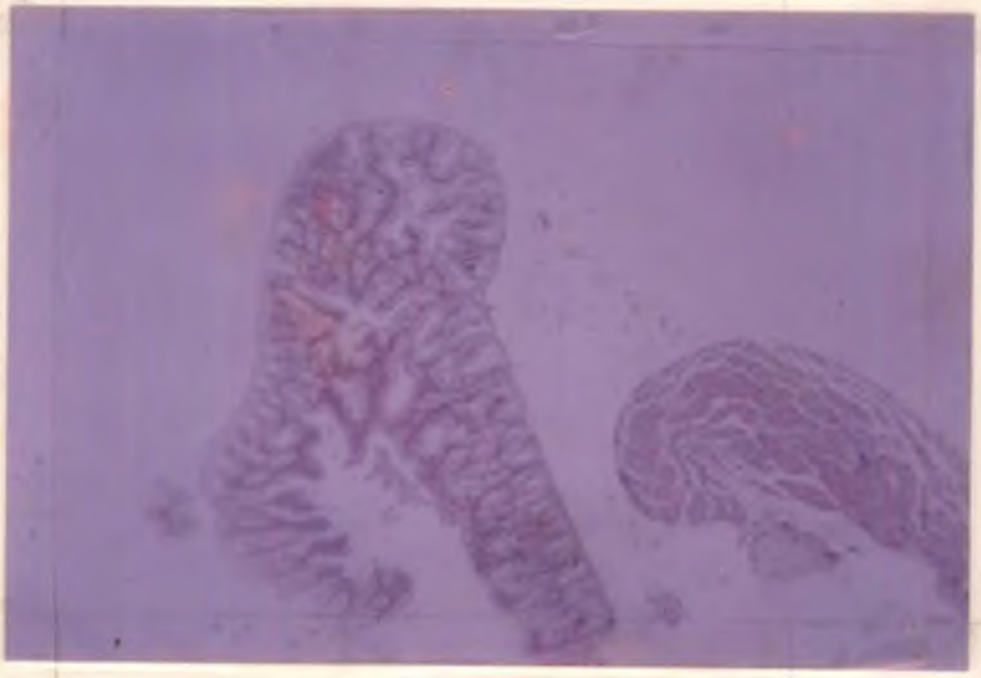
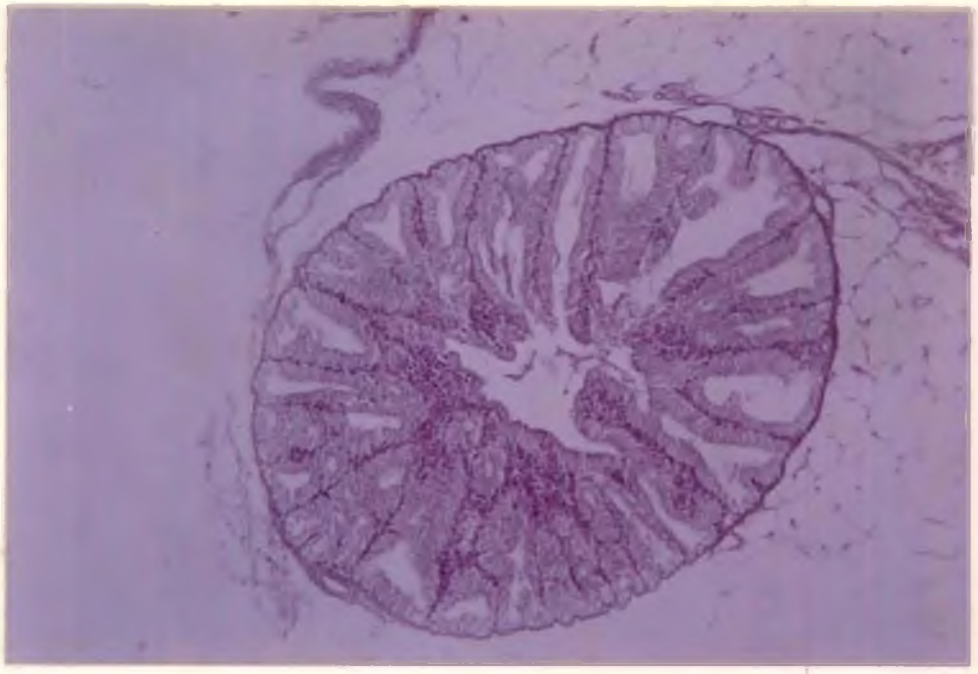


Fig. 15 Harderian gland of the four days old chicken, four days after exposure to IBD vaccine virus showing development of lymphoid foci. H & E x 160.

Fig. 16 Harderian gland of the four days old chicken, four days after exposure to DP vaccine virus showing congestion and infiltration of heterophils and mild degenerative changes of acinar epithelial cells. H&E x 250.



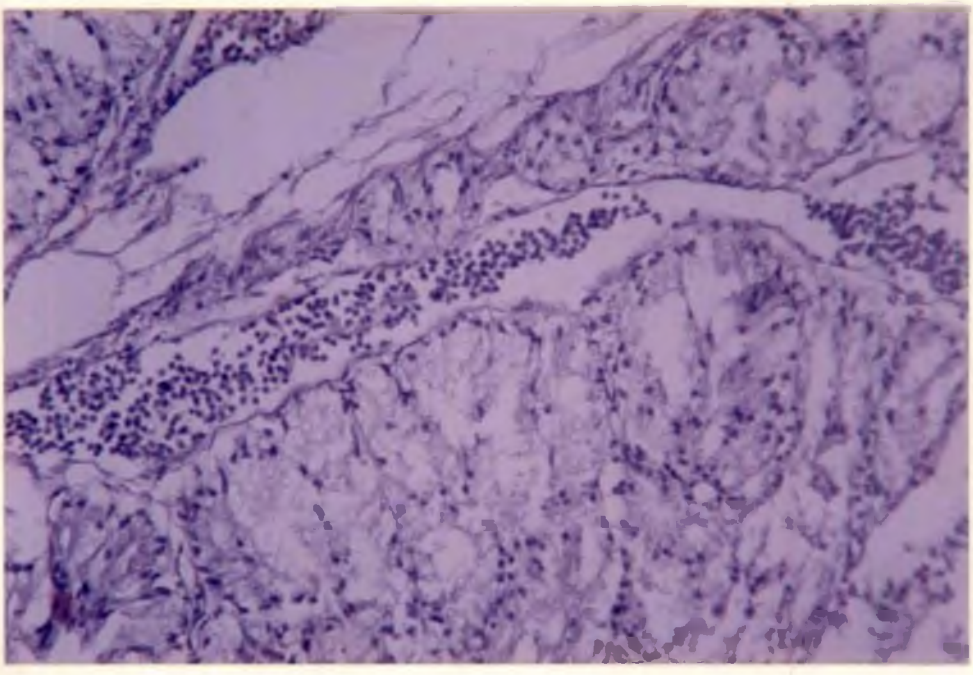
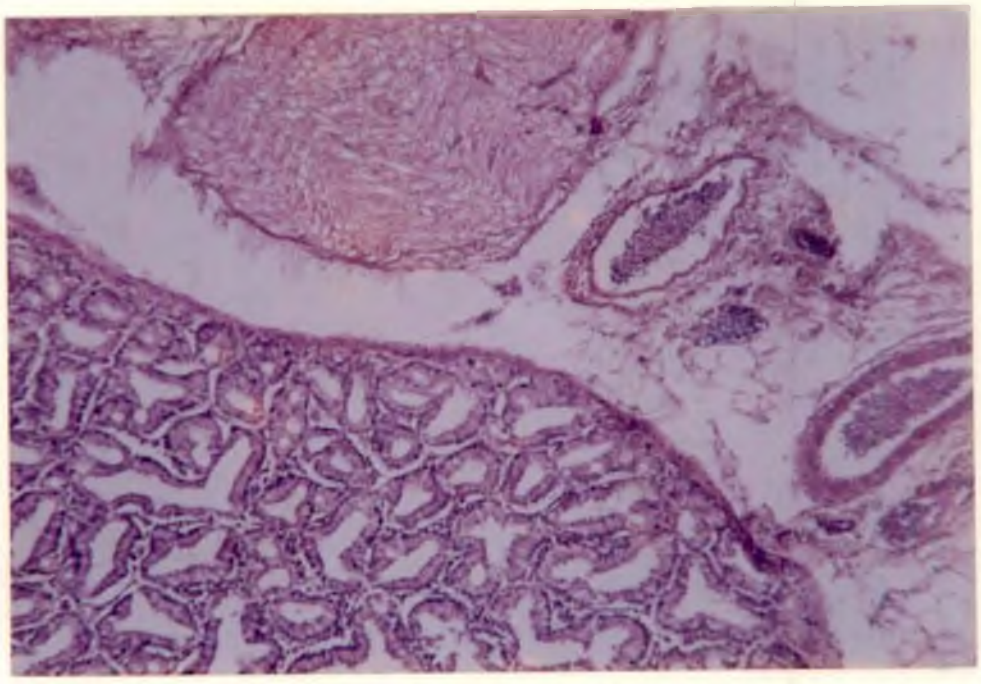


Fig. 17 Harderian gland of the four days old chicken, four days after vaccination with DP vaccine virus showing dilatation of the blood vessels and infiltration of the heterophils in the lobules. H & E x 250.

Fig. 18 Harderian gland of four days control chicken showing Alcian blue positive granules in the interacinar epithelium. Alcian blue x 160.



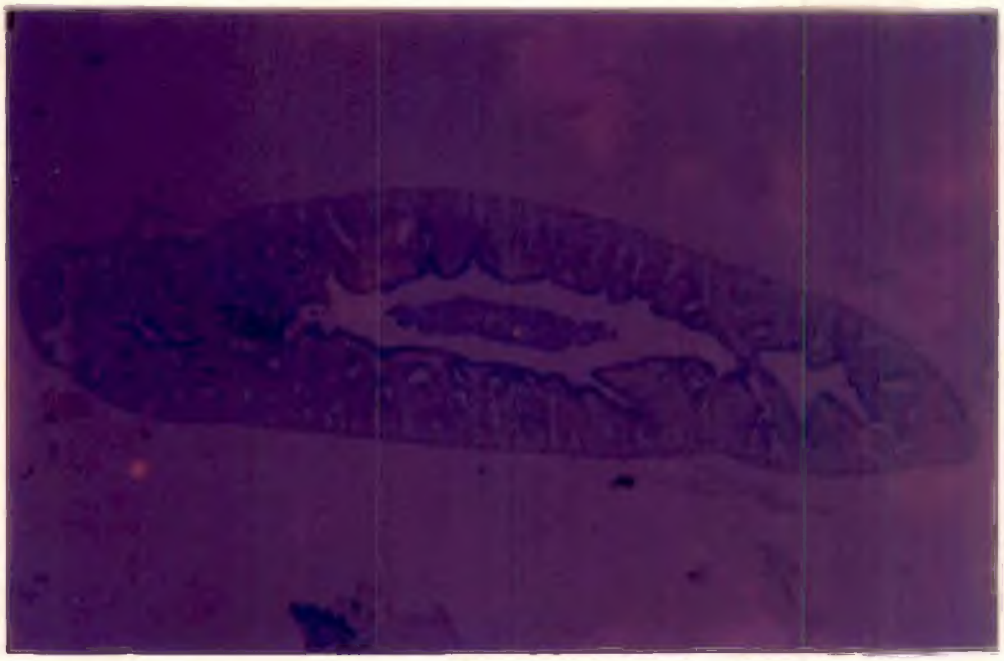
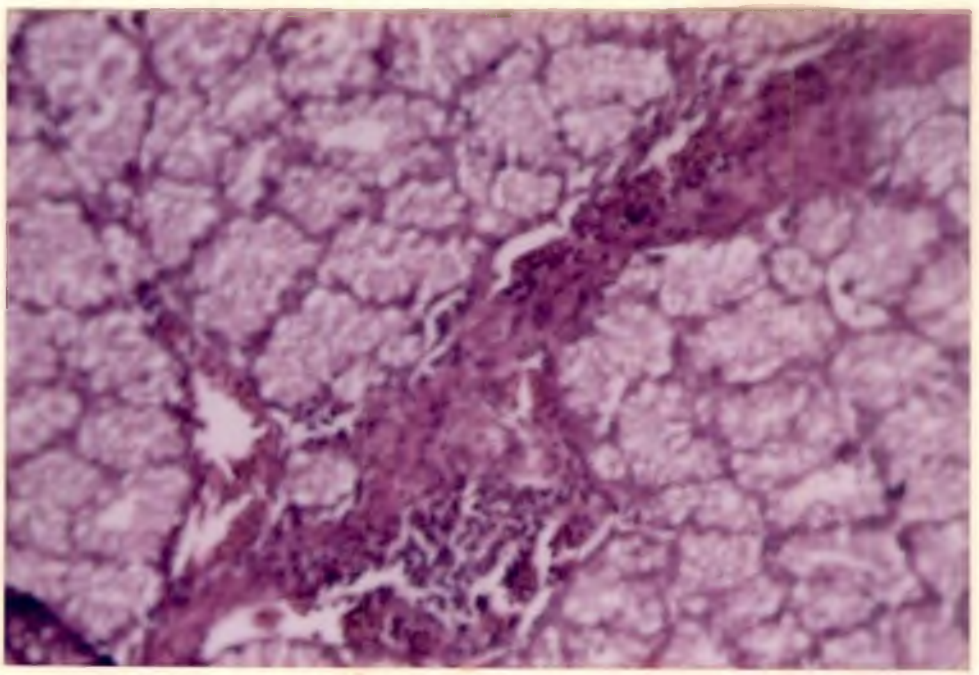


Fig. 19 Harderian gland of eight days old chicken, eight days after exposure to RD vaccine virus showing thickening of the stroma and infiltration of heterophils. H & E x 250.

Fig. 20 Harderian gland of eight days old chicken, eight days after vaccination with RD vaccine virus showing accumulation of plasma cells in the follicular area. Methylgreen pyronine x 1000.



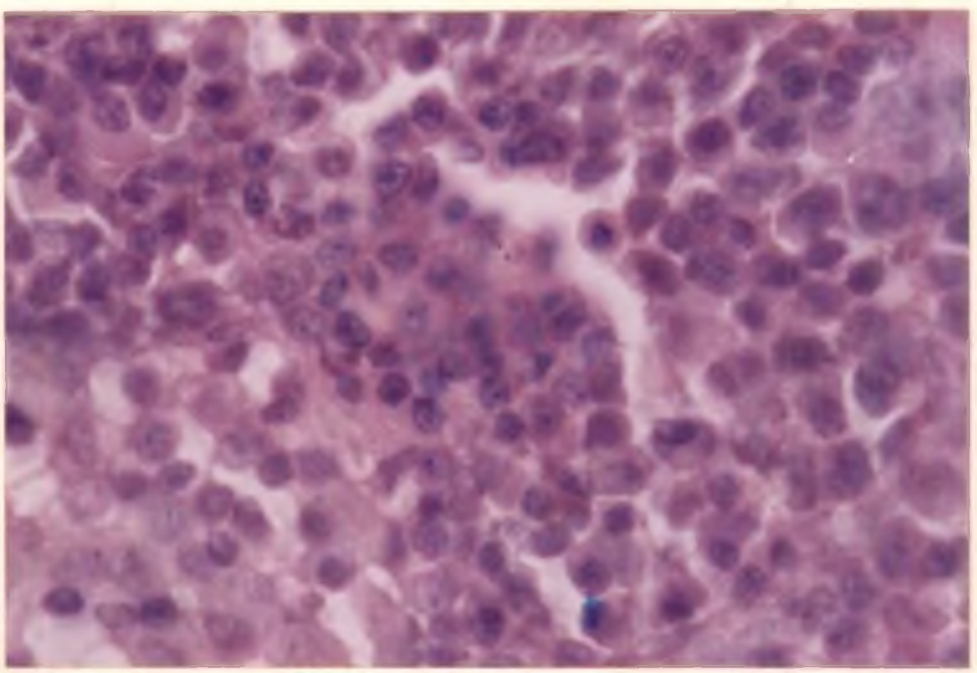
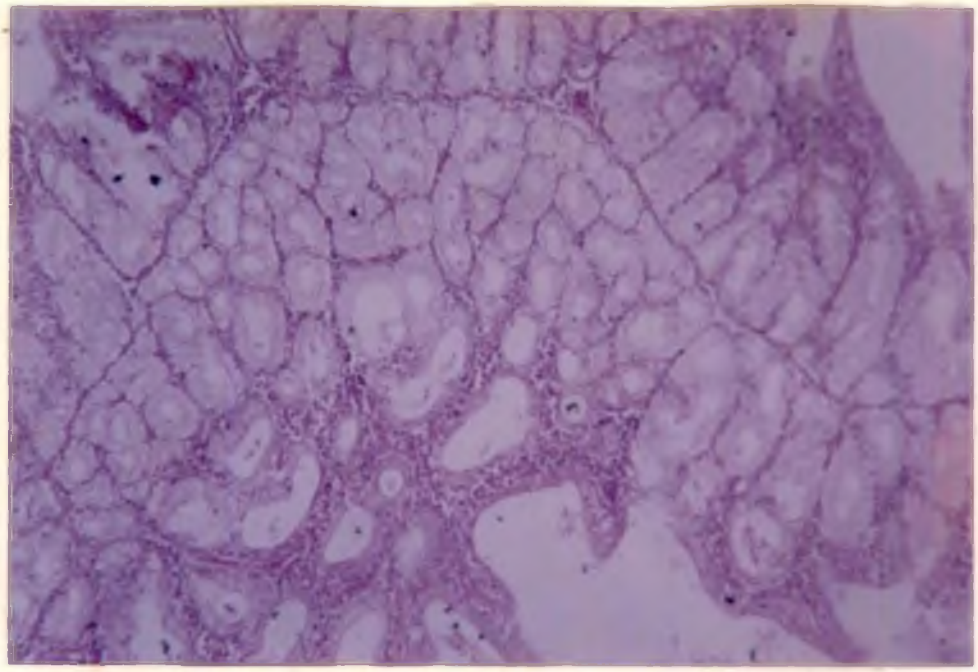


Fig. 21 Harderian gland of eight days old chicken, eight days after vaccination with IBD vaccine virus showing enlarged lymphoid foci. H & E x 250.

Fig.22 Harderian gland of eight days old chicken, eight days after exposure to IBD vaccine virus showing congestion of the interlobular blood vessels, mild infiltration of heterophils and vacuolation of epithelial cells. H & E x 250.



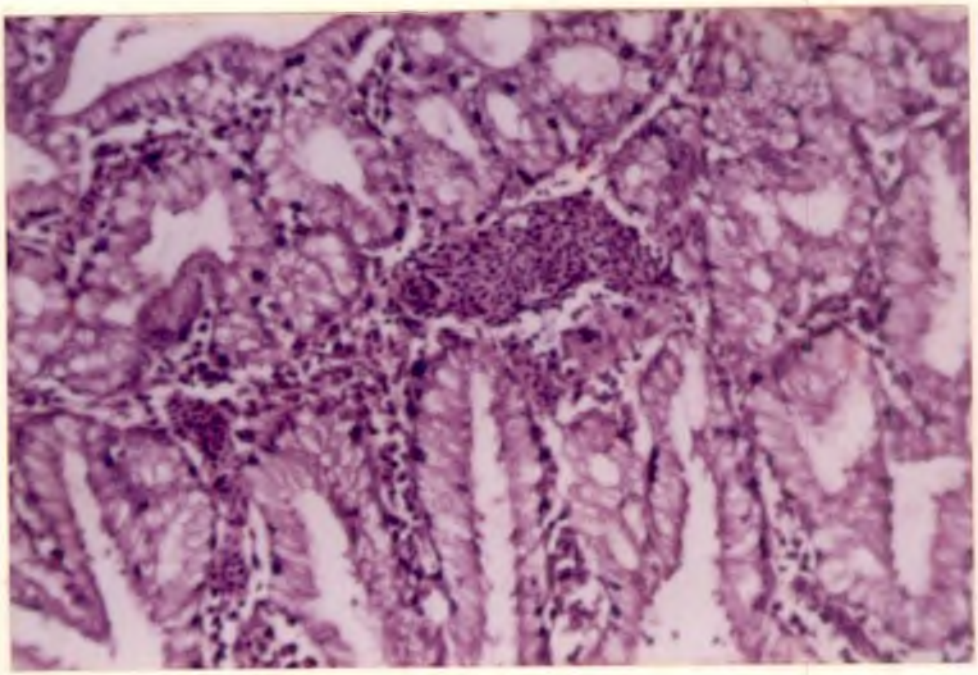
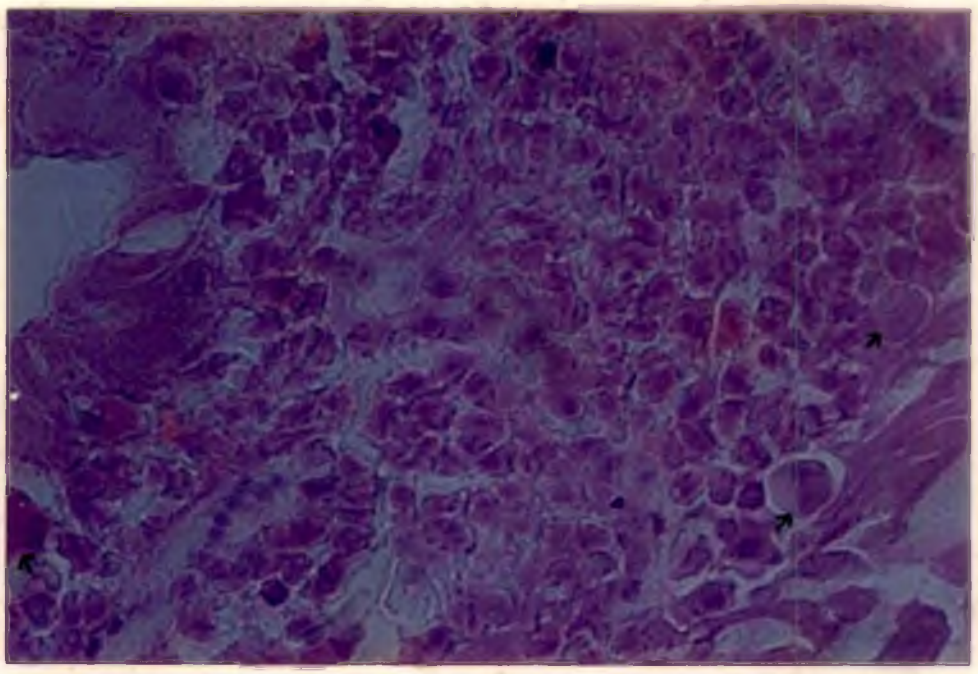


Fig. 23 Harderian gland of eight days old chicken, eight days after inoculation with DP vaccine virus showing increase in plasmablasts, plasma cells and appearance of Russell body (arrow). H & E x 1000.

Fig. 24 Harderian gland of eight days old chicken, eight days after exposure to DP vaccine virus showing severe infiltration of plasma cells. H & E x 1000.



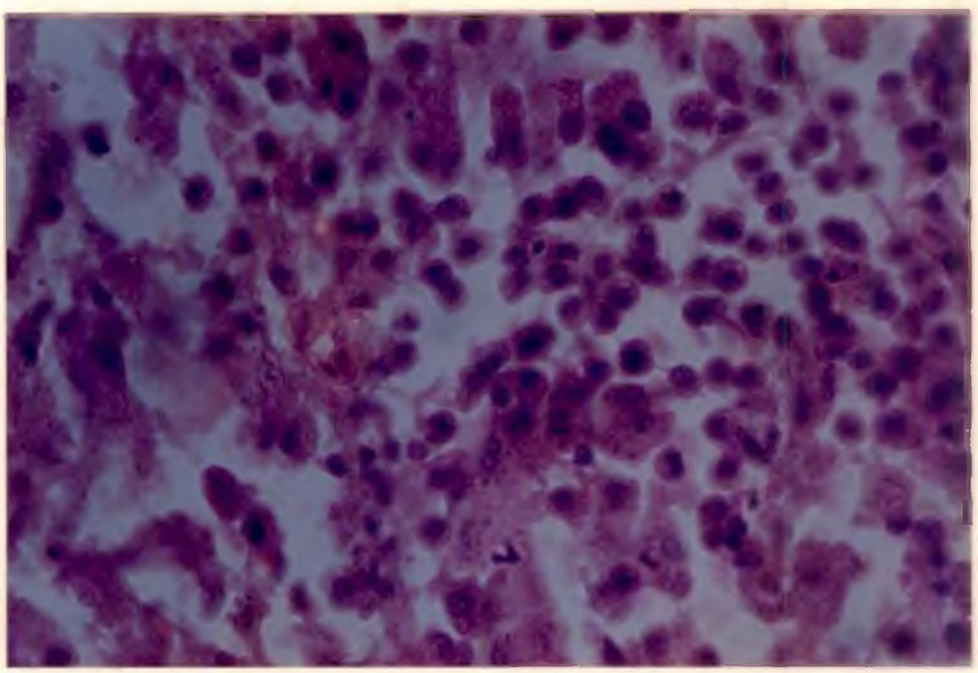
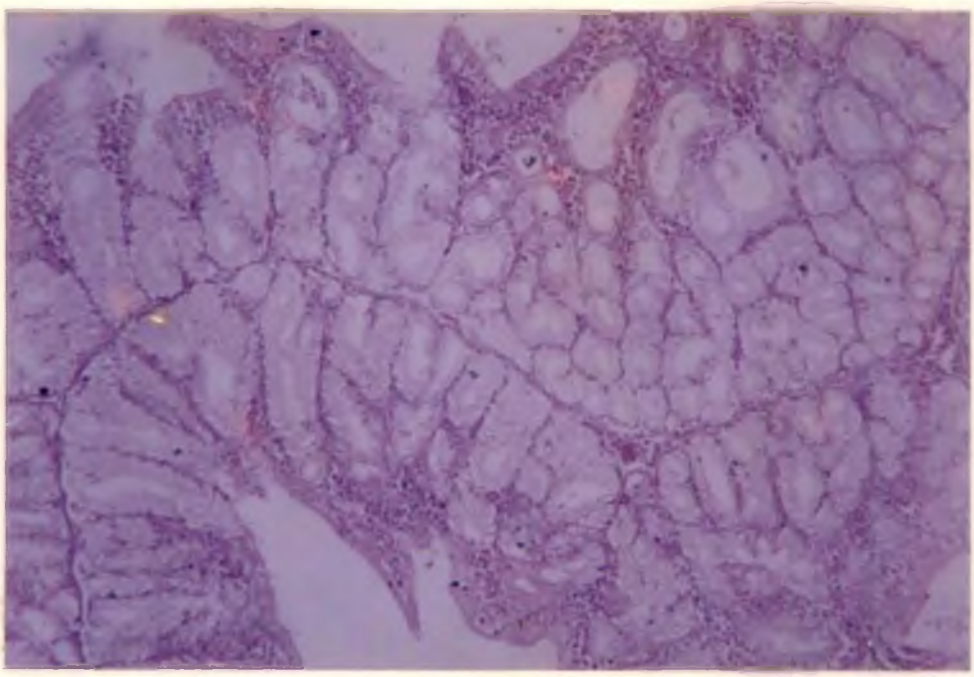


Fig. 25 Harderian gland of eight days old chicken, eight days after exposure to DP vaccine virus showing the follicular area densely packed with plasma cells and lymphoid cells. H & E x 160.

Fig. 26 Harderian gland of eight days old chicken, eight days after exposure to DP vaccine virus showing infiltration of heterophils and vacuolation of epithelial cells. H & E x 450.



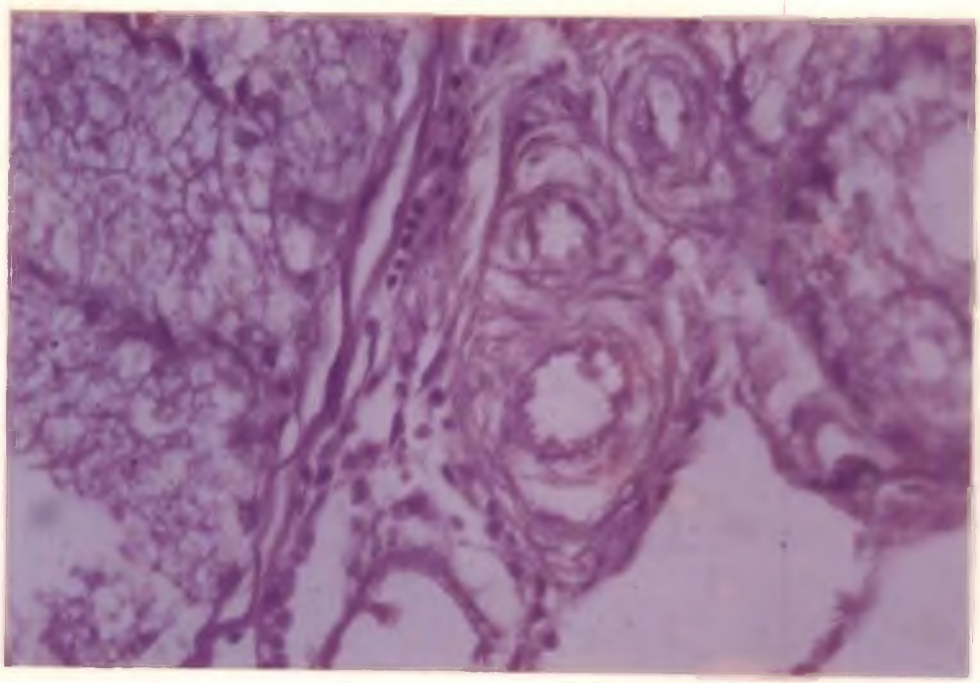
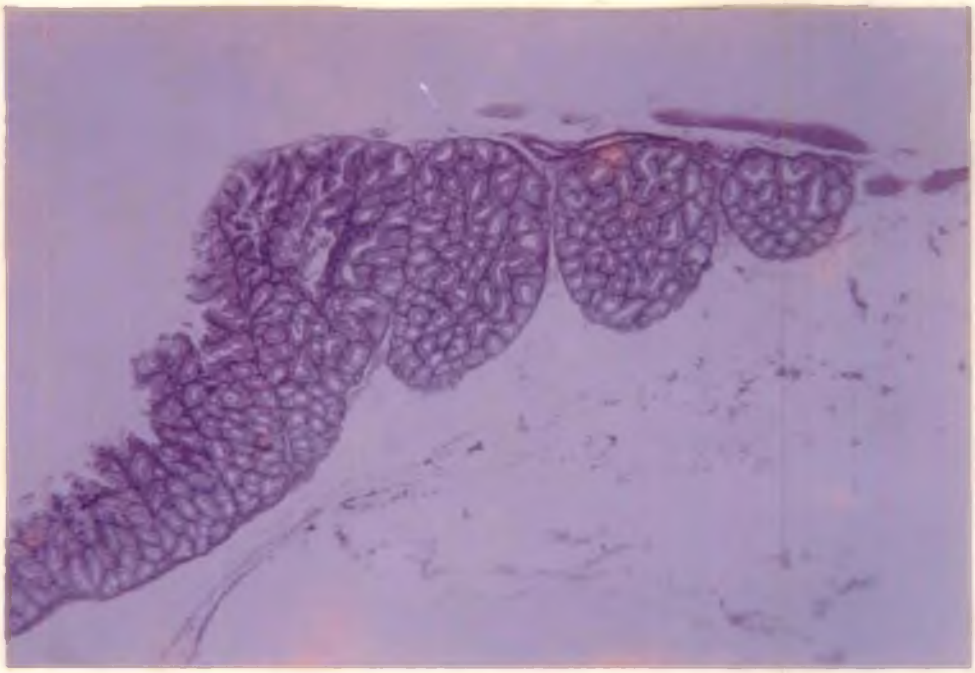


Fig. 27 Harderian gland of eight days old control chicken showing very few lymphoid foci. H & E x 160.

Fig. 28 Harderian gland of eight days old control chicken showing abundant Alcian blue positive granules in the acinar epithelium. Alcian blue x 250.



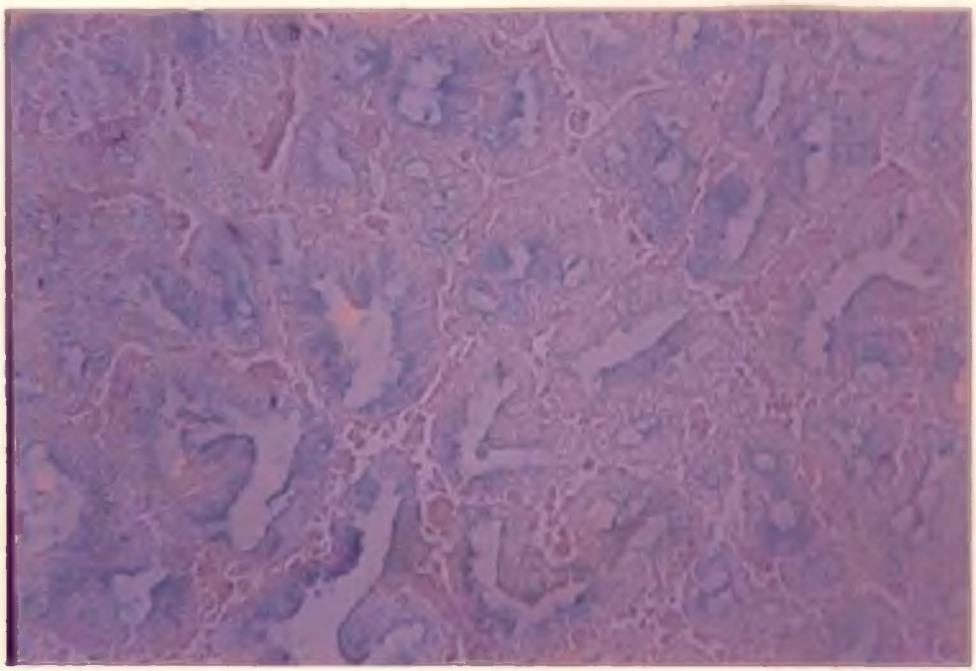
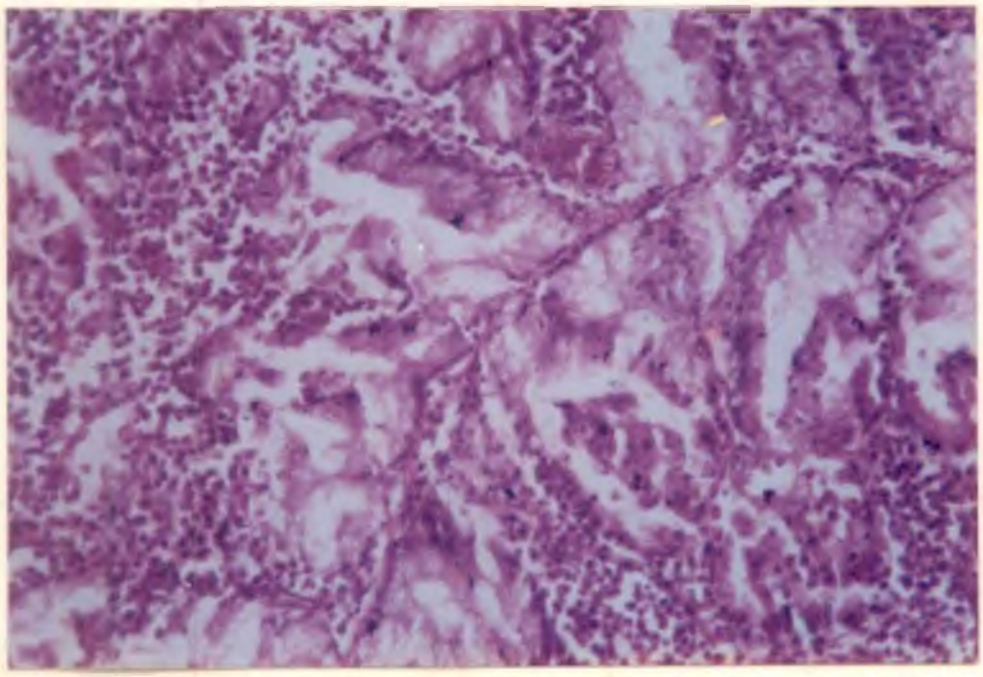


Fig. 29 Harderian gland of twelve days old chicken, twelve days after exposure to RD vaccine virus showing larger number of Russell bodies (arrow) in the interlobular area. H & E x 1000.

Fig. 30 Harderian gland of the twelve days old chicken, twelve days after exposure to RD vaccine virus showing severe infiltration of plasma cells and exfoliation of the epithelial cells. H & E x 160.



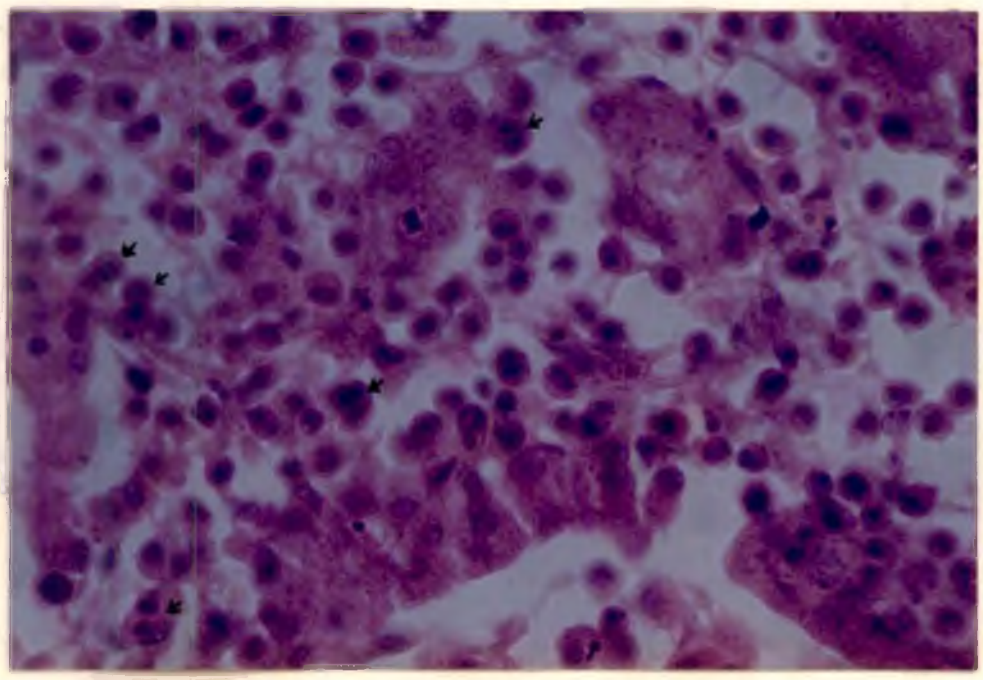
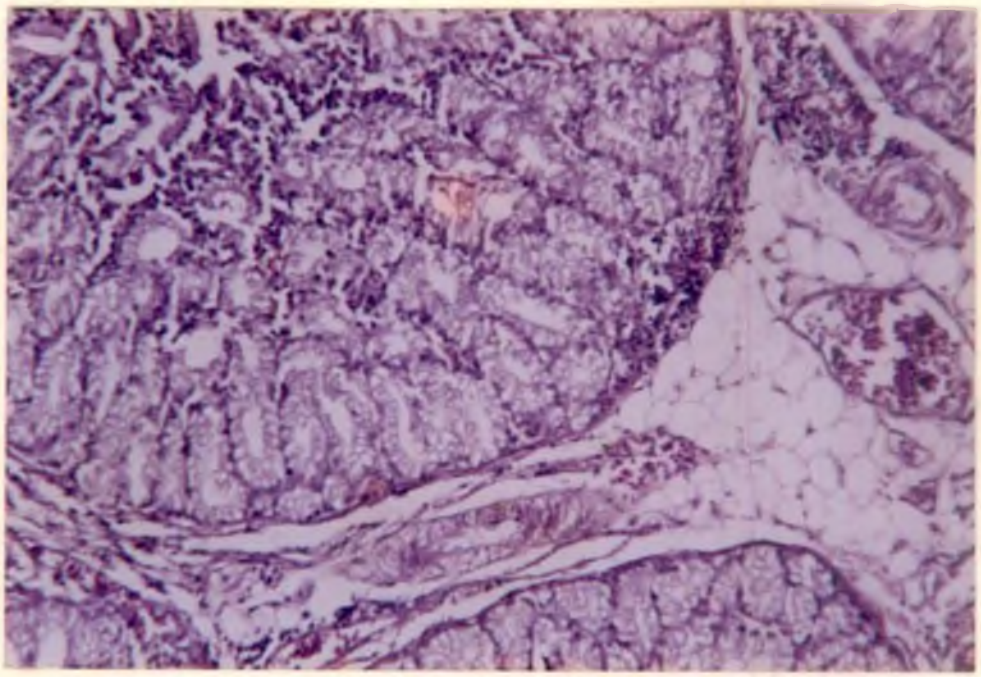


Fig. 31 Harderian gland of the chicken twelve days old, twelve days after exposure to IBD vaccine virus showing degenerative changes of the epithelial cells and increased vascularity of the stroma. H & E x 250.

Fig. 32 Harderian gland of twelve days old chicken, twelve days after exposure to IBD vaccine virus showing severe infiltration of plasma cells in the interstitial tissue. H & E x 1000.



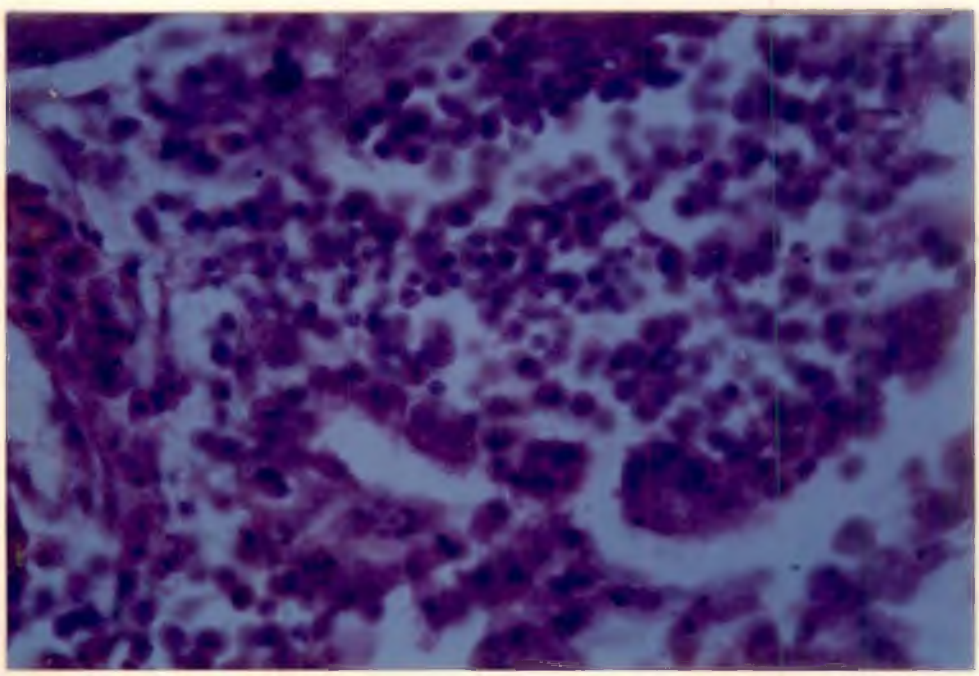
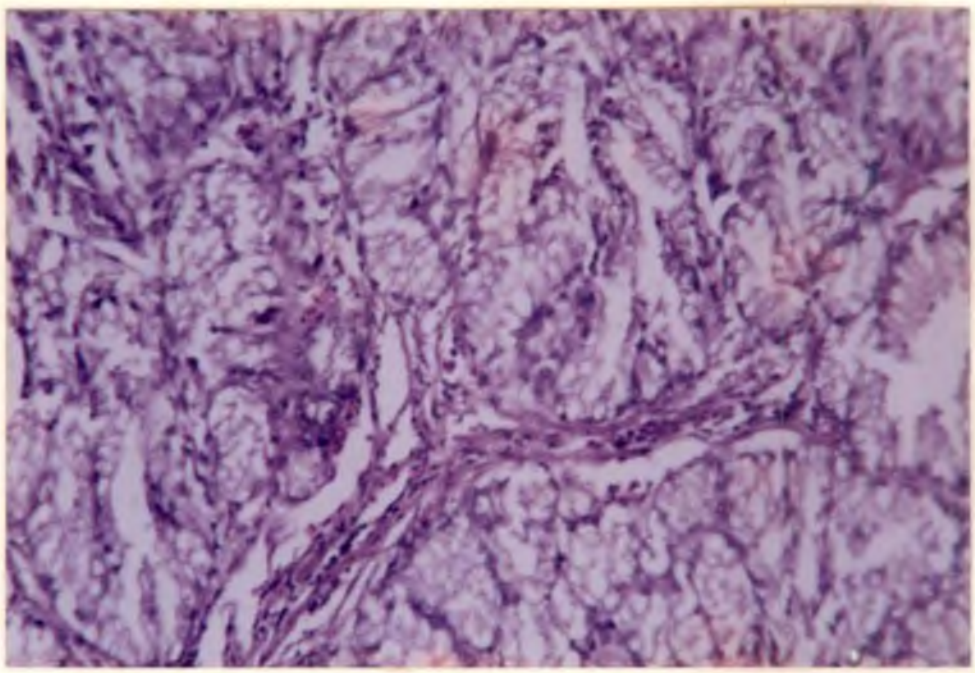


Fig. 33 Harderian gland of twelve days old chicken, twelve days after inoculation with IBD vaccine virus showing thickening of fibrous tissue and infiltration of heterophils. H & E x 250.

Fig. 34 Harderian gland of twelve days old chicken, twelve days after inoculation with IBD vaccine virus showing thickening of fibrous tissue and infiltration of heterophils. H & E x 250.



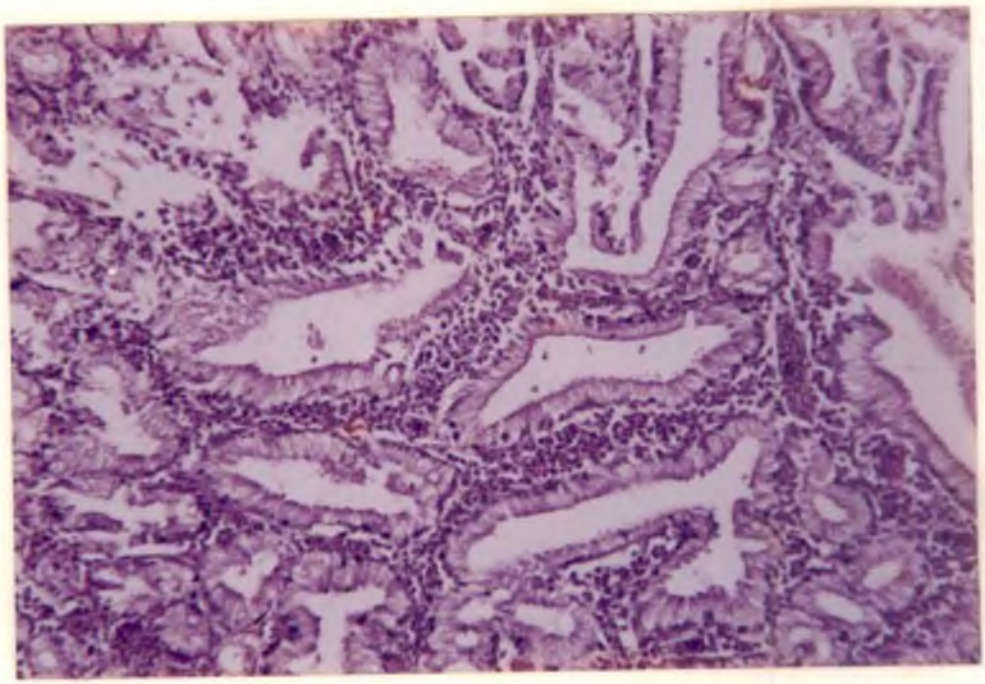
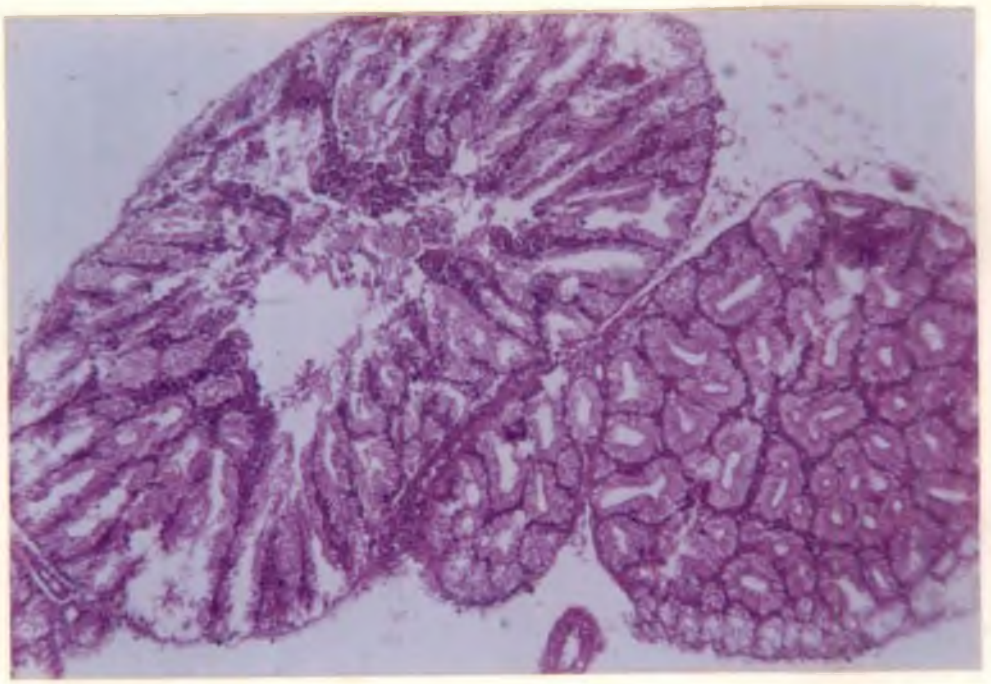


Fig. 35 Harderian gland of twelve days old chicken, twelve days after exposure to DP vaccine virus showing degenerative changes of epithelial cells and lymphocytes. H & E x 160.

Fig. 36 Harderian gland of twelve days old chicken, twelve days after vaccination with DP vaccine virus showing severe infiltration of plasma cells. H & E x 160.



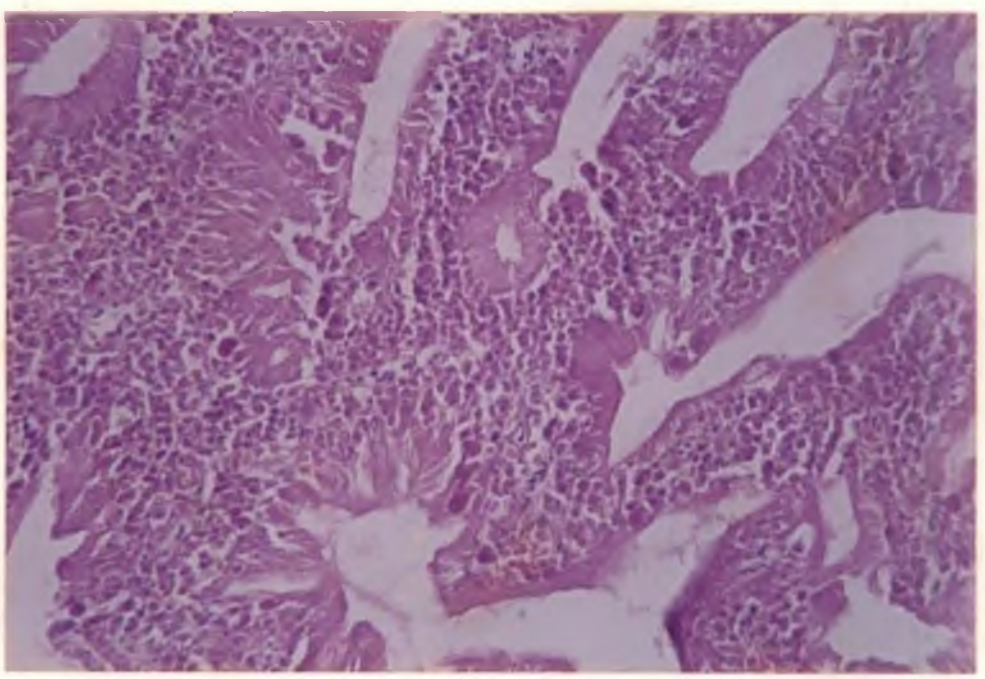
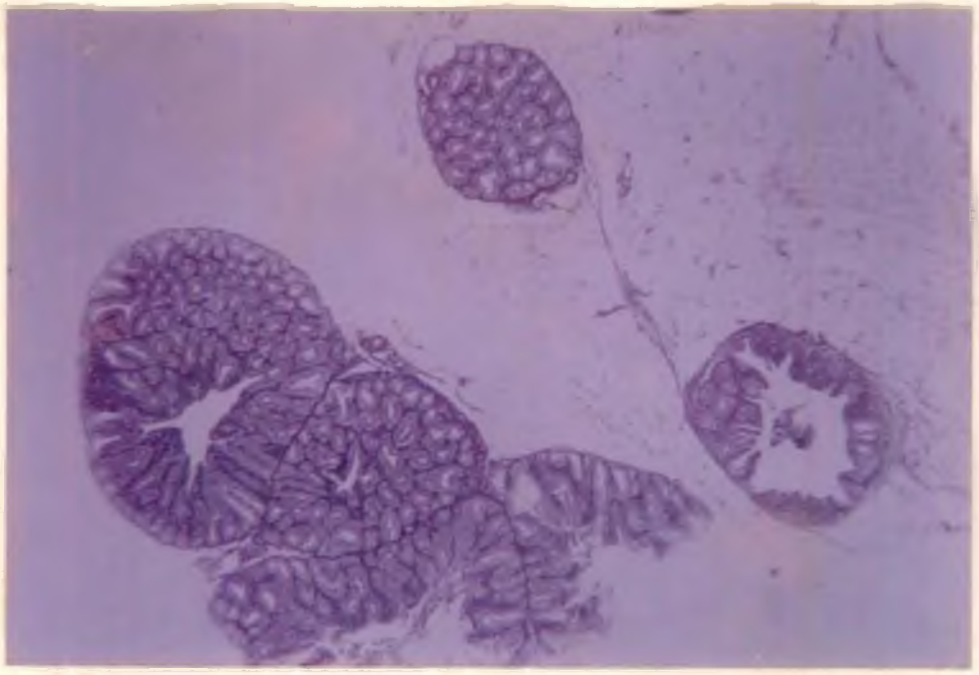


Fig. 37 Harderian gland of the twelve days old control chicken showing few lymphoid foci. H & E x 160.

Fig. 38 Harderian gland of four days old duckling, four days after exposure to DP vaccine virus showing mild degenerative changes in the epithelial cells. H & E x 250.



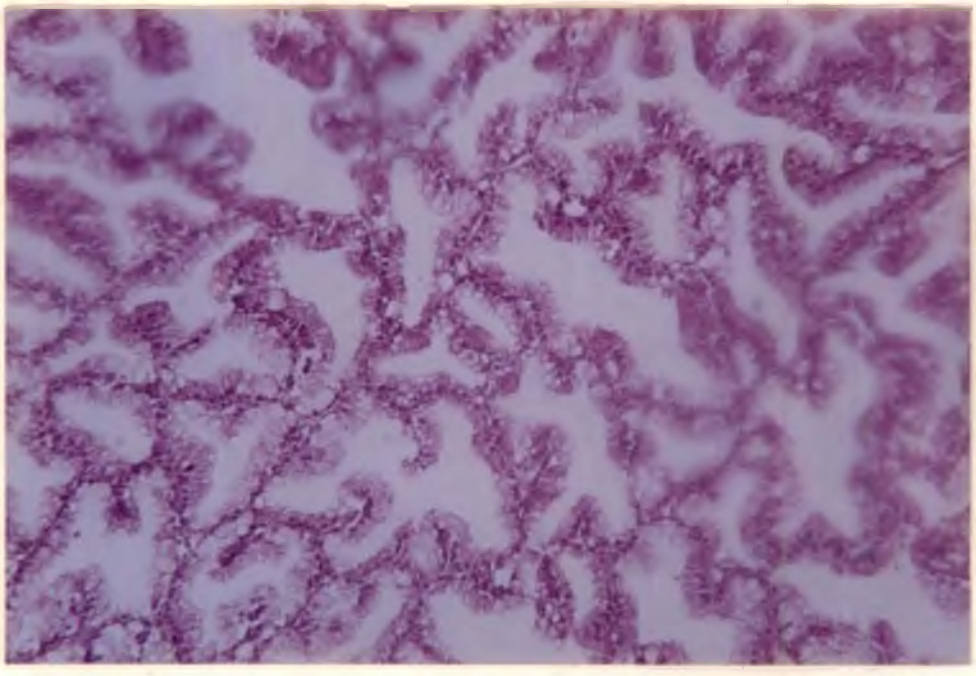
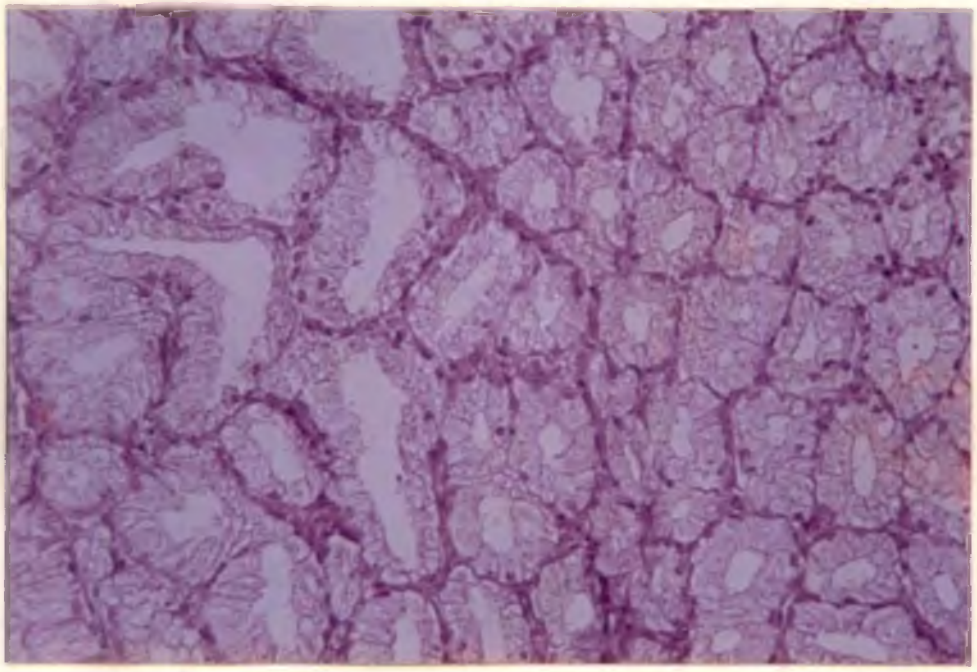


Fig. 39 Harderian gland of eight days old duckling, eight days after exposure to RD vaccine virus showing vacuolation of follicular epithelial cells and mild thickening of fibrous tissue. H & E x 250.

Fig. 40 Harderian gland of eight days old duckling, eight day after exposure to IBD vaccine virus showing degenerative changes in the epithelial cells. H & E x 250.



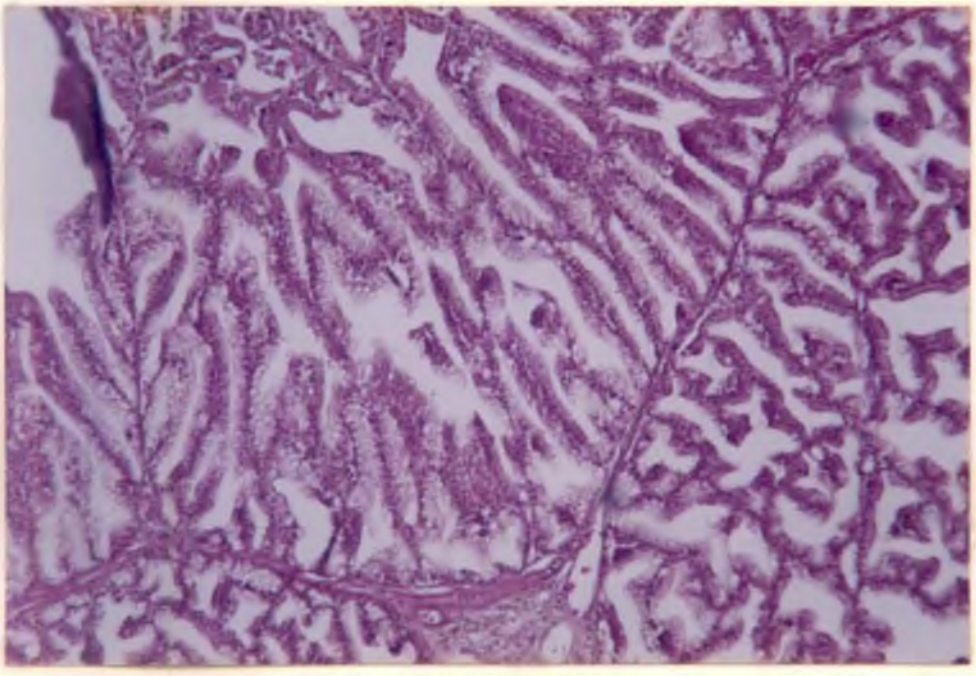
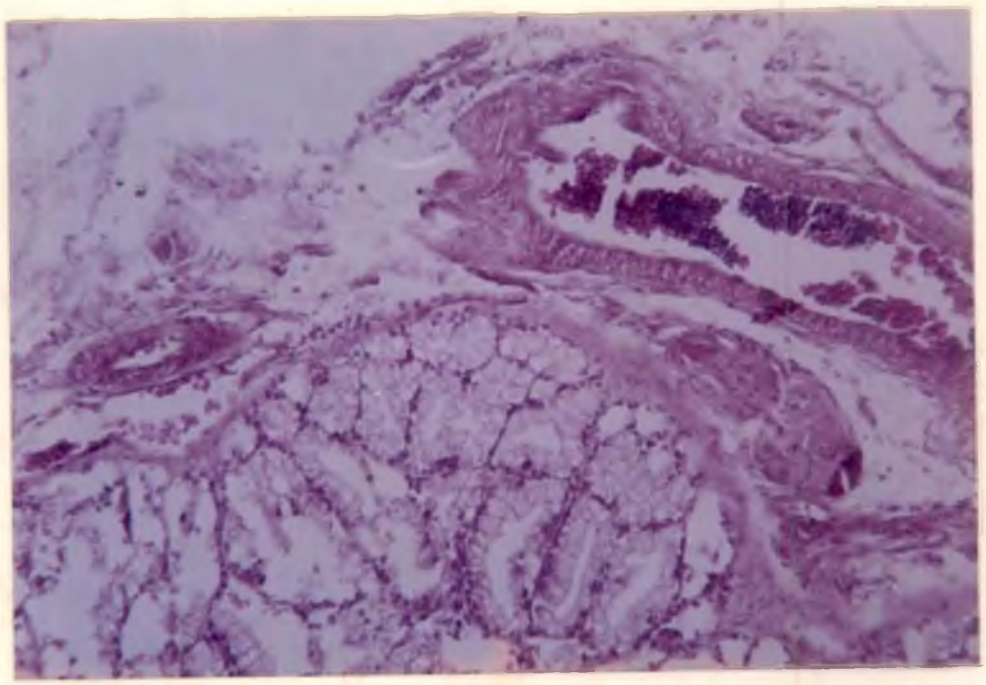


Fig. 41 Harderian gland of eight days old duckling, eight days after exposure to DP vaccine virus showing dilatation of the capillaries and increased vascularity of the stroma. H & E x 400.

Fig. 42 Harderian gland of eight days old control duckling showing Alcian blue positive granules in the tubular epithelium. Alcian blue (pH 2.5) x 160.



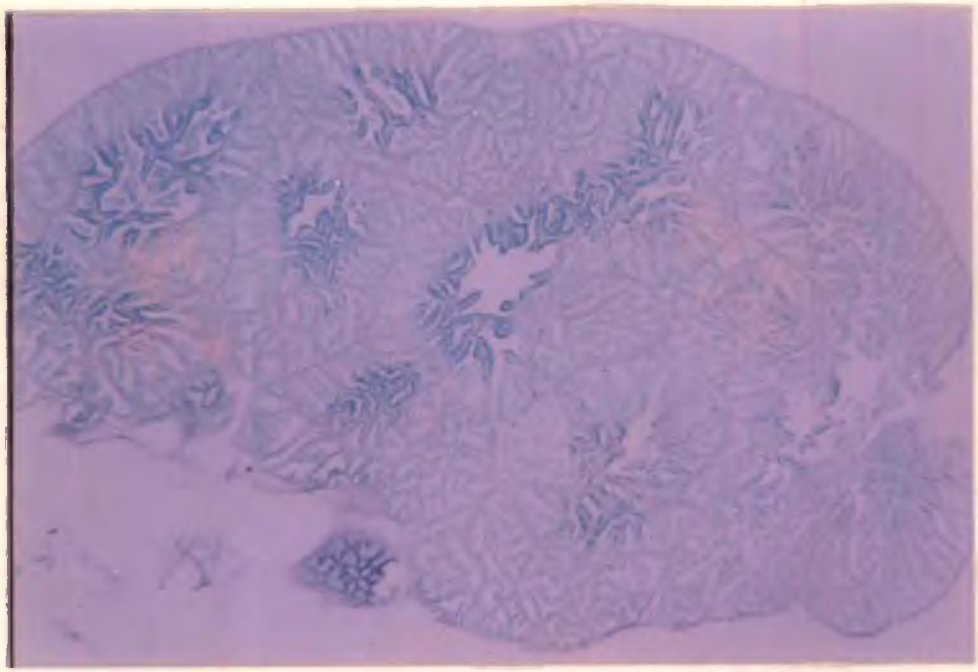
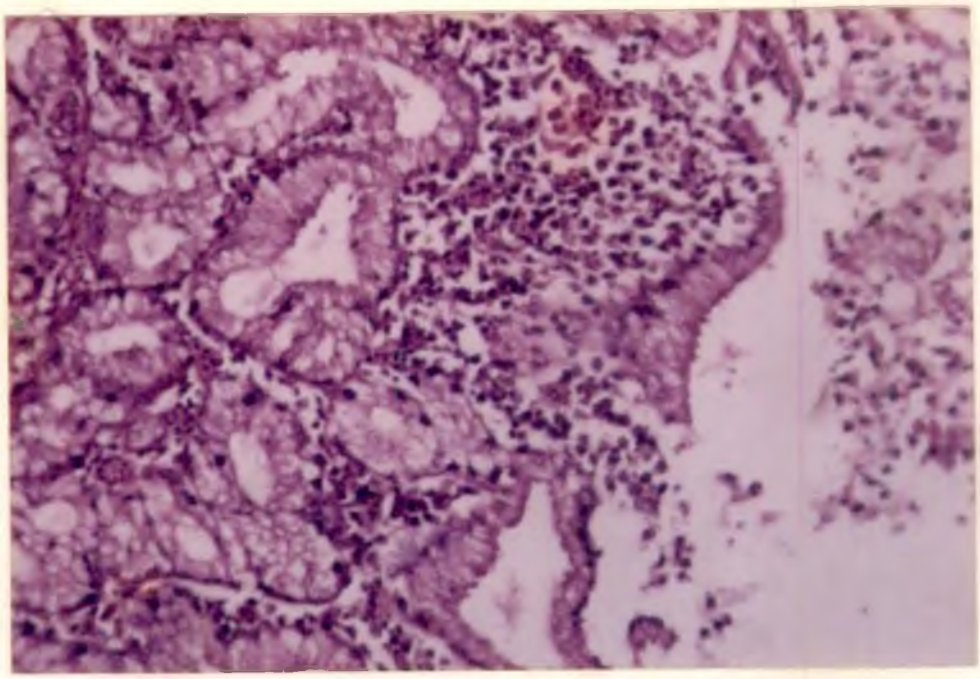


Fig. 43 Harderian gland of twelve days duckling, twelve days after exposure to RD vaccine virus showing lymphoid and plasma cell foci. H & E x 250.

Fig. 44 Harderian gland of twelve days old duckling, twelve days after inoculation with IBD vaccine virus showing thickening of the fibrous tissue, congestion of blood vessels and vacuolation of epithelial cells. H & E x 250.



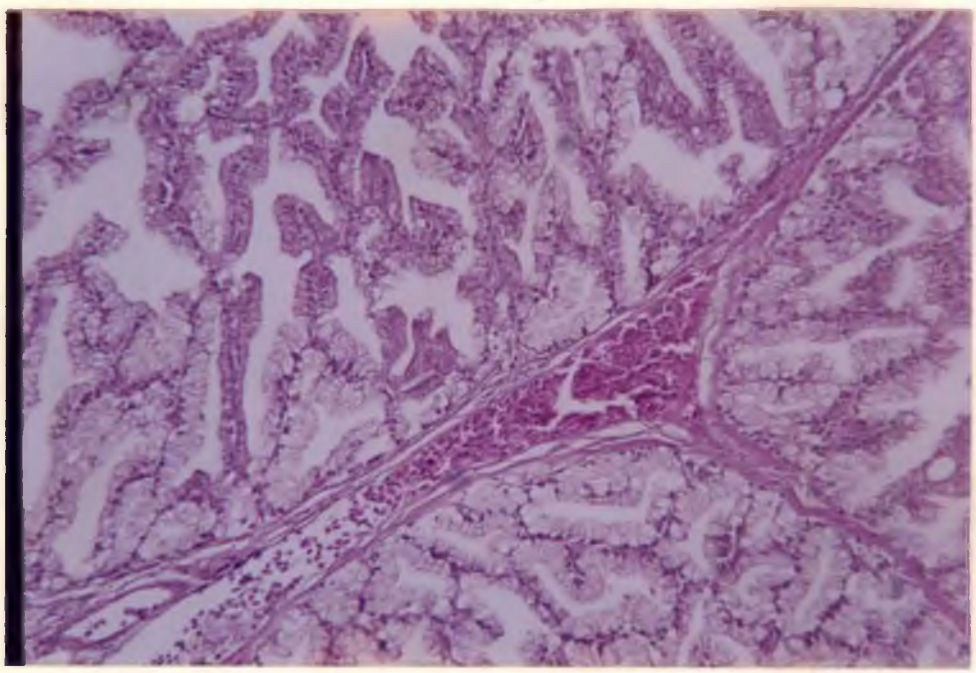
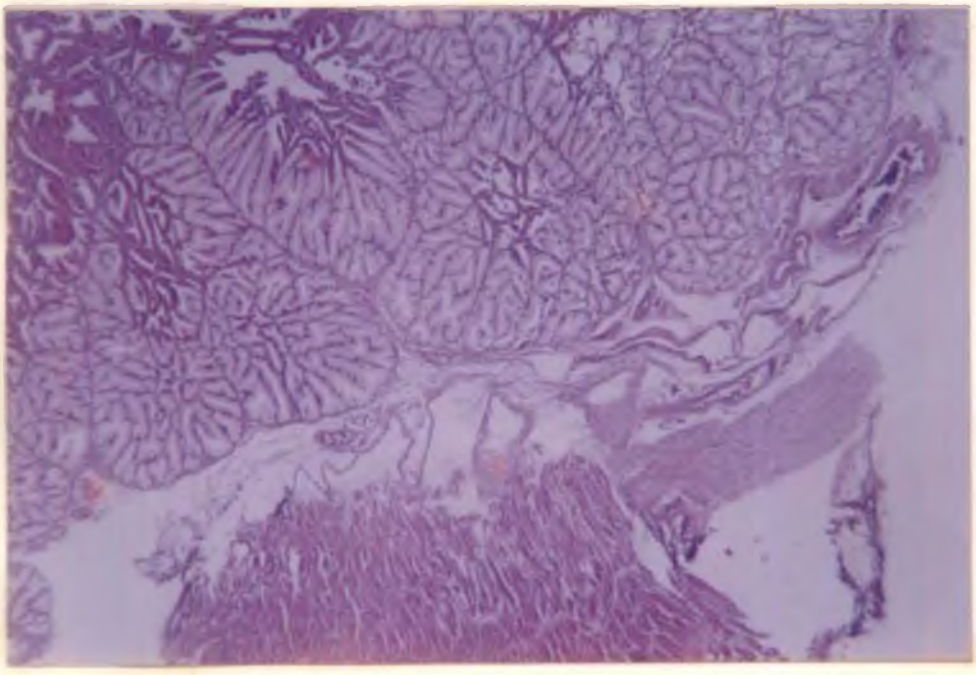


Fig. 45 Harderian gland of the twelve days old duckling, twelve days after stimulation with IBD vaccine virus showing congestion of blood vessels and vacuolation of the epithelial cells. H & E x 250.

Fig. 46 Harderian gland of twelve days old duckling, twelve days after exposure to DP vaccine virus showing increased vascularity of the stroma and dilatation of the capillaries. H & E x 250.



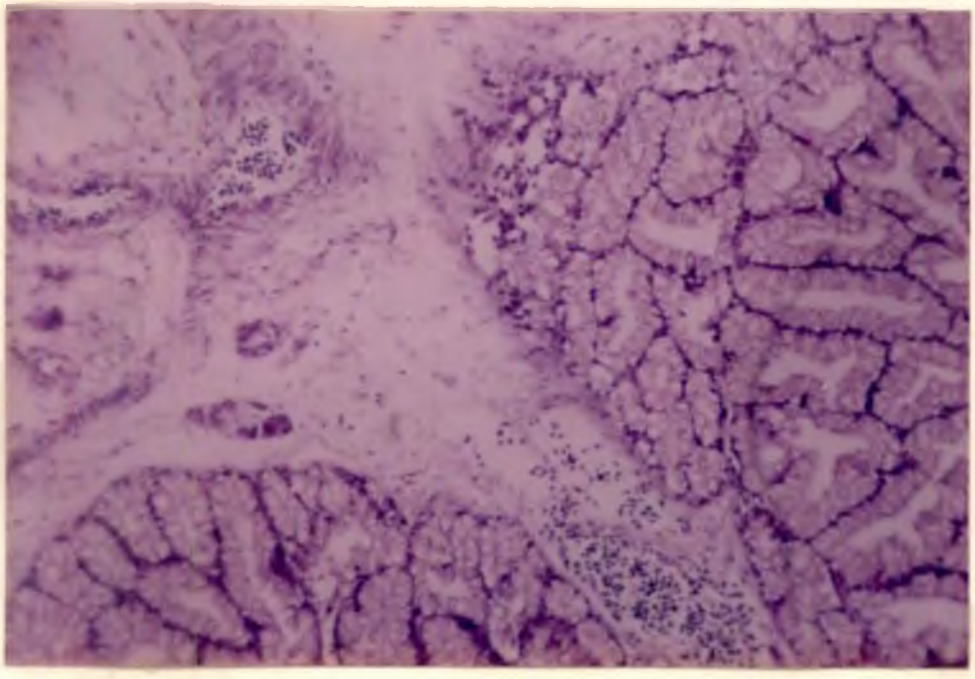
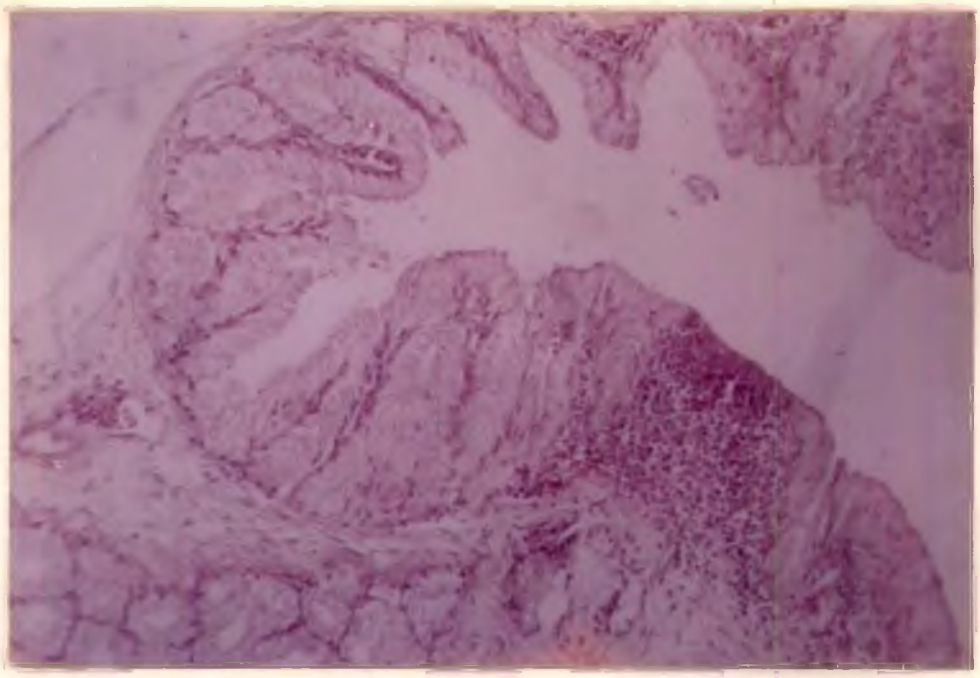


Fig. 47 Harderian gland of twelve days old duckling, twelve days after vaccination with DP vaccine virus showing development of lymphoid foci in the follicular area. H & E x 250.

Fig. 48 Harderian gland of twelve days old duck, twelve days after exposure to DP vaccine virus showing infiltration of few plasma cells and macrophages. H & E x 1000.



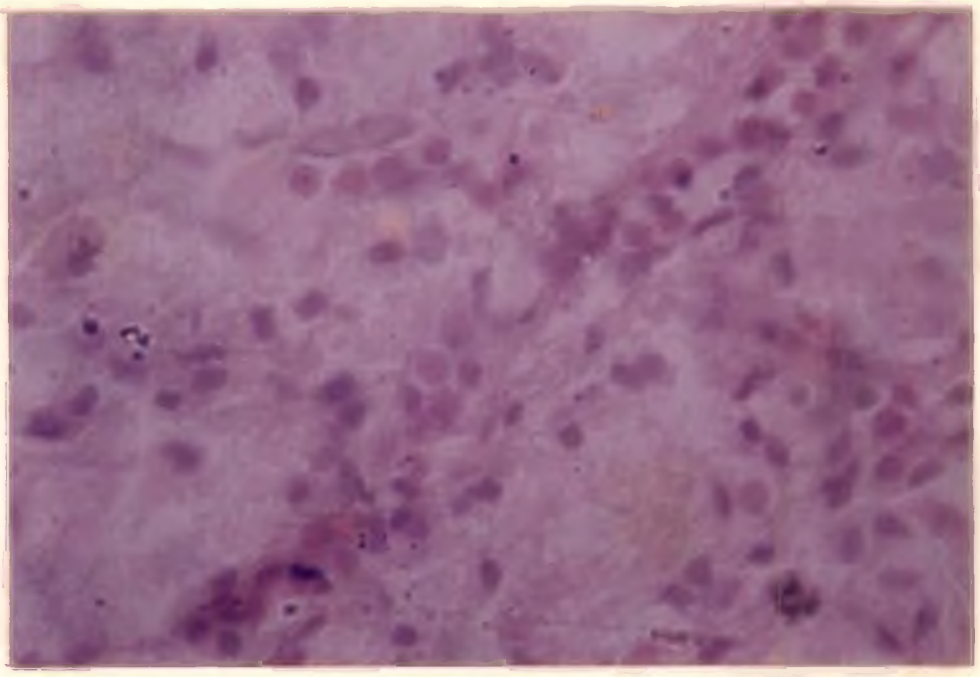
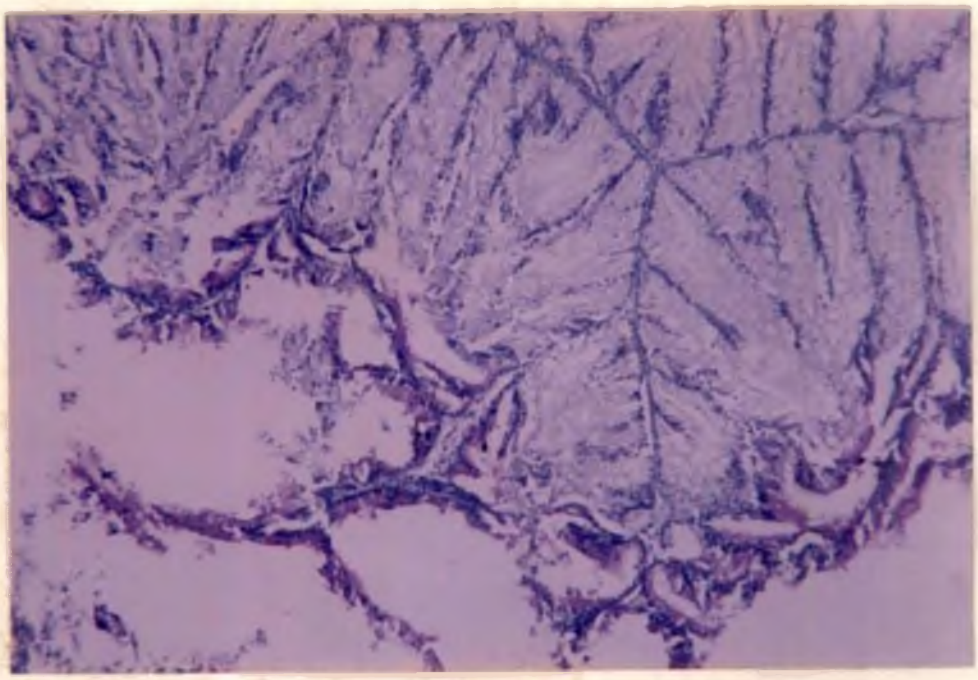
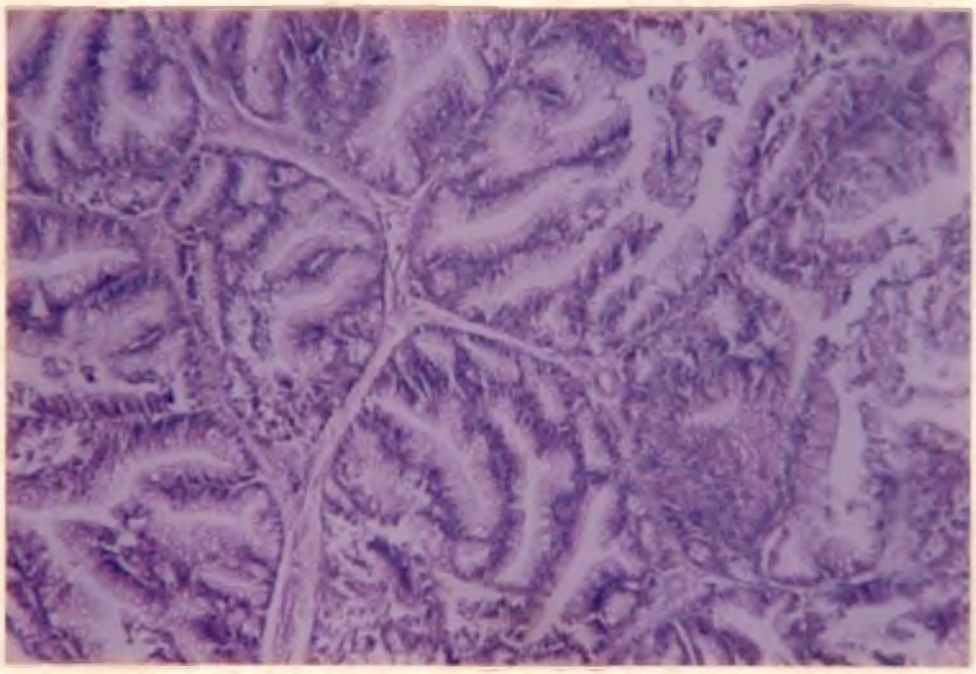


Fig. 49 Harderian gland of twelve days old control duckling showing metachromasia of the epithelial cells. Toluidine Blue x 250.

Fig. 50 Harderian giand of twelve days old control duckling showing metachromasia of the tubular epithelial cells. Thionine stain x 400.





DISCUSSION

5. DISCUSSION

In order to evaluate the role of the Harderian gland in the immunopathological response an investigation was undertaken taking chicken and duck as models and RD, IBD and Duck plague vaccines as antigens. The sequential cellular response following antigenic stimulation was chartered and the significance was clarified.

Following antigenic stimulation there was significant and pronounced increase in the weight of the Harderian gland in the chicken and duck. This proves to show that antigens have been delivered at the gland and there has been stimulatory response characterised by quantitative increase and qualitative improvement in the cellular constituents and components in the gland. The histological studies also supported and confirmed the findings and established that there was significant increase in the cellular response following antigenic stimulation.

However, there was no significant difference in the response between different antigenic responses. The geometrical parameters of the gland following antigenic stimulation were also documented and analysed, and they also correlated with the increase in the weight of the gland. In this context it may be pointed out that this data documented relating to the weight and geometrical parameters of the Harderian gland following antigenic stimulation have not been reported earlier for comparative analysis and confirmation.

The Harderian gland of the chicks following intraocular antigenic stimulation with RD vaccine and IBD vaccine on histological examination revealed marked vacuolation of the tubular epithelium, plasma cell infiltration and lymphiod foci. The formation of lymphoid foci and plasma cell infiltration demonstrated that there was immunostimulatory response in the Harderian gland on the day four itself .

On the 8th day the gland also showed moderate heterophilia. The lymphoid foci and plasma cell response were more when compared to the 4th day. The epithelial cells of the acini and tubules were normal. Increased vascularity was observed in the stroma and Russell bodies were evident.

On the 12th day there was progressive and significant increase in the formation of lymphoid foci and plasma cell response. Hyperemia of the follicles was significant. However, vascularization of the stroma had decreased. Plasma cells containing Russell bodies cells were significantly increased in the interacinar tissue. Appearance of macrophages was evident at this stage. Some individual plasma cells displayed nuclear pyknosis or karyorrhexis. There was increase in the number of capillaries and they were dilated and groups of erythrocytes were seen filling them.

The above observations are in agreeement with the findings of Davelaar and Kouwenhoven (1976), Powell *et al.* (1979), Survashe *et al.* (1979), Davelaar and Kouwenhoven (1980) and Toro *et al.* (1996). They observed increase in the plasma cell count, lymphoid foci, epithelial cell vacuolation and hyperaemia of the follicle when they inoculated the vaccine strains of RD, IBD and IB in the day old chicks and observed the histological changes on the subsequent days. It is significant to note that plasma cell count and lymphoid foci were progressively increasing following antigenic stimulation on the 4th, 8th and 12th day of the investigation in both chicken and ducks.

The tissue response as indicated by the histological changes in the Harderian gland was significant and it was clarified that the lymphoid and plasma cell response were the hall marks of the response. This is to be so since for the humoral immune response and for the synthesis of immunoglobulins plasma cells are required. The

Russell bodies represent the synthetic activity of the plasma cells and is considered as a histological marker for this. The infiltration of heterophils on the 8th and 12th day has to be considered as a secondary response to the presence of partly disintegrating antigen and mild degenerative changes in the gland associated with the immunobiological response.

The above observations are in confirmity with those observed by Davelaar and Kouwenhoven (1976), Burns (1977) and Survashe *et al.* (1979). They considered the lymphoid activity, plasma cell response and Russell body formation as the hall marks of the immune response and the involvement of the heterophils as the secondary response of the Harderian gland of the chicken following ocular antigenic challenge.

It may also be pointed out that as reported by Survashe and Aitken (1978) the presence of heterophils in the gland in chicks and ducks following antigenic stimulation may serve to transport antigen and to stimulate immune response.

Plasma cell response in the Harderian gland of the chicken and duck was comparable. However, the sequential development of the response in the duck was relatively at a low pace when compared to the chicken. The plasma cell response was evident only on the 12th day in the duck. This would suggest that the Harderian gland of the duck is relatively less efficient in inducing antibody response. Therefore, the immunological response following intraocular vaccination may not yield the same response as in the case of chicken.

The antibody response in the RD vaccinated chicken and duck revelaed a titer of 8 on the 12th day by HI test but no antibody response was detected on the 4th and 8th day.

The histological changes in the Harderian gland of the RD vaccinated chicken revealed pronounced lymphoid and plasma cell response on the 12th day with progressive increase of plasma cells containing Russell bodies. Hyperemia of the follicles and heterophilia were also evident. This was consistant with the antibody response.

The Harderian gland of the RD vaccinated group of ducks showed mostly, tubular epithelial cell degeneration and very few plasma cells and no Russell body on the 4th, 8th and 12th day. The Plasma cell count was more in the RD vaccinated chicks on the 8th and 12th day than the IBD vaccinated chicks. This observation clarified that the RD vaccine virus might stimulate an increase of plasma cells in the Harderian gland and conferred better protection than IBD vaccine by the intraocular route of vaccination. It may also be construed that RD virus is a better antigen.

The above observations in chicks challenged with RD are in accordance with the findings of Aitken *et al.* (1976), Survashe *et al.* (1979) and Avram and Bucor (1982). They recorded increase in the plasma cell count and lymphoid foci following ocular challenge in chicks by the vaccine strain of RD. However, there are no published reports on the efficacy of RD vaccination in ducklings by the intraocular route.

It is very reasonable to surmise that formation of Russell bodies in the plasma cells are the result of ocular antigenic stimuli leading to the biosynthesis of immunoglobulins by the plasma cells. Investigation on the secretory mechanisms of plamsa cell of the chicken Harderian gland by Stobbe (1960), Besis (1961) and Melchers (1971) suggested that Russell bodies are formed by the rapid accretion of immunoglobulins within the plasma cell as result of antigenic challenge and these cells are found to be comparatively larger than the other plasma cells.

White (1974) suggested that the Russell body formation in the Harderian gland of the chicken was a response to ocular antigenic challenge that activated the gland.

The presence of intraepithelial lymphocytes and macrophages in the intralobular tissue following antigenic stimulation in the chicks and ducks suggest that they may have a role in the transport of antigens. Similar observation was made of Del Cacho *et al.* (1992) in the chicken after the antigenic stimulation with RD. They also pointed out that these cells have a role in the antigen processing.

It would also appear that the epithelial cells of the Harderian gland also have a stimulatory effect on cells of the lymphoid series as in the case of Bursa and the thymus, where the epithelial cells have been demonstrated to have a stimulatory role on the immunocompetent cells. This lymphoepithelium allows selective sampling of local antigens and facilitates presentation of those antigens to the cells of the immune system. The hypertrophy and vacuolation of the epithelial cells observed following antigenic stimulation are morphological alterations indicating a functional role. This has to be investigated in detail and confirmed by further studies.

Macrophages were observed in relatively large numbers in the interstitial tissue following antigenic stimulation, along with other cellular population in chicks but not in ducks. These macrophages are reported to be present to process the antigen and deliver to the lymphocytes and there by mediate cellular response. This observation would also point out that the Harderian gland of the duck is less efficient in processing the antigen when compared to the chicken.

Mueller *et al.* (1971) confirmed the importance of the gland in the fowl and suggested that all the cells in the Harderian gland are primarily capable of responding to the local antigenic stimulation.

According to the report of Delcacho *et al.* (1992) the ultrastructural features of Harderian gland in the chicken showed fibronectin, an electron dense material secreted and released from the myofibroblast which played a main role in the antigenic regulation and processing. This feature has not been reported in the duck.

Davinson *et al.* (1996) observed that the Harderian glamd of the chicken consistered of 80 percent of B cell and 20 percent T cell population. The finding supported the concept of immunologic function of the Harderian gland which was similar to the function of the thymus on the one hand and bursa of Fabricius on the other hand, thus implying that the gland might be active in humoral immunity.

Evidence has been postulated by Sundick *et al.* (1973) that lymphoid cells of the chicken Harderian gland are mainly bursal dependent and involved in humoral immune response.

Significant increase in the plasma cell number and enlargement of the lymphoid foci on the 4th, 8th and 12th day following antigenic stimulation, clarified the immunopotency of the Harderian gland in the chicken but this was not so much significant in the case of ducks. It would, therefore, appear that through local adminnistration of the antigen at the Harderian gland of the ducks may not have the same result as in the case of chicken. However, it is worthwhile to investigate whether any immunostimulators when administered with the antigen can enhance the response.

The conjunctival location of the gland may provide exposure to the environmental antigens including microorganisms to the Harderian gland. The antigen could gain access by up take through specialized Harderian gland epithelial cells and entry in to macrophage -lymphoid population.

Initial antigenic exposure through conjunctival assoicated lymphoid tissue (CALT) with subsequent localisation of plasma cells in the Harderian gland provides

"homing up" mechanism specific for the protection of ocular and upper respiratory surfaces in the chicken and ducks.

Intraocular antigenic stimulation in the chicks was found to be the best route for antigenic stimulation. By this route antigen gets regulated, amplified and processed by the Harderian gland plasma cells and protects the birds from IB,ILT and other upper respiratory tract diseases.

This is true in the case of chicken. It is also true in the case of ducks which are aquatic in nature and their constant movement in water and contact of the eye and conjuctiva with the water will certainly lead to exposure of the Harderian gland to various antigens.

In ducks the literature describing the immunological response of the Harderian gland and response due to the antigenic stimulation are lacking and this would appear to be the first report.

Intraocular stimulation gives beneficial results in ducks also since other lymphoid structures like CALT, lachrymal gland may associated with the function. This investigation, has therefore, helped to focus light on the need for probing into the usefulness of intraocular vaccination in ducks also, as a vaccination programme against Duck plague as in the case of intraocular RD vaccination in the chicken.

Survashe (1992) suggested that during mass vaccination, ocular vaccination was found to be comparatively more effective in immunological protection in chicken. This may be also true in the case of ducks against respiratory viral pathogens. The local antibody produced by the Harderian gland contributes in a major way to the local immune protection to the oculonasal and oropharyngeal and upper respiratory

tract and they have an obvious relevance to the epidemiology and the control of respiratory viral diseases of chickens.

The main observation of the study is that the paraocular gland, the Harderian in particular, is highly immunologically reactive especially in the chicken but not so much in the case of ducks.

There is, abundant scope to investigate and explore the posibility and assess the efficacy of intraocular vaccination against various infections in ducks.

SUMMARY

6. SUMMARY

The objective of the present study was to evaluate the immunopathological response of the Harderian gland following ocular antigenic stimulation and to clarify the nature and extent of the immunobiological response in the chicken and duck.

Vaccine strains of RD, IBD and DP were administered intraocularly in both eyes of the day old chicks and ducklings. The antibody response in the RD vaccinated birds revealed a titer of 8 on the 12th day by HI test but no antibody response was detected on the 4th and 8th day. The Harderian glands of both species were harvested on the 4th, 8th and 12 th day. The geometrical changes and weight of the gland were recorded and the histological changes were studied and compared. There was significant increase in the weight of the gland and geometrical measurements following antigenic stimulation.

The histological examination demonstrated that there was quantitative increase and qualitative improvement in the cellular constituents and components in the gland.

The formation of the lymphoid foci and plasma cell infitration on the day four following antigenic stimulation in chicks demonstrated that there was immunostimulatory response in the Harerian gland on the day four itself.

There was progressive and significant increase in the formation of lymphoid foci and plasma cell response on the 12th day in the chicken. Plasma cell containing Russell body were significantly increased in the tissue.

The lymphoid activity, plasma cell response and Russell body formation were the hall marks of immune response and the heterophils present were involved in the secondary response of the Harderian gland of the chicken following ocular antigenic challenge. The presence of heterophils in the gland in chicks following antigenic stimulation served to transport antigen and to stimulate immune response.

The Harderian gland of the vaccinated groups of the duck showed mostly tubular epithelial cell degeneration and few plasma cells and no Rusell bodies.

The sequential development of the cellular response in the duck was relatively at a low pace when compared to the chicken . The plasma cell response was evident on the 12th day in the duck. This suggested that the Harderian gland of the duck is relatively less efficient in inducing antibody response.

The formation of Russell body in the plasma cells was considered as the result of ocular antigenic stimuli leading to the biosynthesis of immunoglobulins by the plasma cells.

The epithelial cells of the Harderian gland were suggested to have stimulatory effect as in the case of the thymus and bursa, where the epithelial cells have been demonstrated to have a stimulatory role in the immunopotent cells. The hypertrophy and vacuolation of the epithelial cells observed following antigenic stimulation were the morphological alterations indicating a functional response.

Significant increase in the plasma cell number and enlargement of the lymphoid foci on the 4th, 8th and 12th day following antigenic stimulation, clarified the immunopotency of the Harderian gland in the chicken, but this was not so much significant in the case of duck. It would therefore, appear that through local

administration of the antigen at the Harderian gland the duck may not respond the same way as in the case of chicken.

Initial antigenic exposure through conjunctival associated lymphoid tissue (CALT) and subsequent localisation of the plasma cells in the Harderian gland provided strong humoral immune mechanism specific for the protection of the ocular and upper respiratory tract surfaces in the chicken and duck.

There was no difference in the histological response to various antigens used.

The scope for further investigation on the immunological response of the paraocular glands in the ducks was indicated.

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PATHOLOGY OF THE HARDERIAN GLAND IN CHICKEN AND DUCK

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ABSTRACT OF A THESIS

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ABSTRACT

The lymphoid tissue of the upper respiratory tract includes paraocular and paranasal lymphoid structures as well as some lymphoid accumulations in the pharynx and larynx. However, of all these paraocular lymphoid tissue, the Harderian gland is highly immunopotent.

The role of the Harderian gland in the immunopathological response was evaluated taking chicken and duck as models and RD, IBD and Duck plague vaccines as antigens. The sequential cellular response following ocular antigenic stimulation was clarified.

Significant increase in the plasma cell number, enlargement of the lymphoid foci and Russell body formation following ocular antigenic stimulation were the hall marks of the immune response of the Harderian gland of the chicken but this was not so much significant in the case of ducks.

Harderian gland was highly reactive especially in the chicken but not so much in the case of ducks. Intraocular vaccination was found to be comparatively more effective in immunological protection in chicken. It would therefore, appear that through local administration of the antigen at the Harderian gland the duck may not respond in the same way as in the case of chicken.

It was pointed out that the local antibody produced by the Harderian gland contributed in a major way to the immunological defence at the oculonasal and oropharyngeal areas and it has an obvious relevance to the epidemiology and the control of respiratory viral diseases in the avian species.