IMMUNOGLOBULINS IN DUCKS AND ROLE OF BURSA OF FABRICIUS IN THEIR PRODUCTION

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "IMMUNOGLOBULINS IN DUCKS AND ROLE OF BURSA OF FABRICIUS IN THEIR PRODUCTION" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

(G. KRISHNAN NAIR)

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CERTIFICATE

Certified that this thesis, entitled "IMMUNOGLOBULINS IN DUCKS AND ROLE OF BURSA OF FABRICIUS IN THEIR PRODUCTION" is a record of research work done independently by Sri. G. Krishnan Nair 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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LIST OF ABBREVIATIONS

ABC	: Antibursal serum administered, uninoculated
ABS	: Antibursal serum
ABSR	: Antibursal serum administered and SRBC inoculated
ABSt	: Antibursal serum administered and *S. typhimurium* inoculated
ASS	: Ammonium sulphate solution
Bx	: Bursectomy
CSR	: Non-bursectomised and SRBC inoculated
CSt	: Non-bursectomised and *S. typhimurium* inoculated
Cy	: Cyclophosphamide
CyC	: Cyclophosphamide treated, uninoculated
CySR	: Cyclophosphamide treated and SRBC inoculated
CySt	: Cyclophosphamide treated and *S. typhimurium* inoculated
DE	: Day of embryonation
HBx	: Hormonal bursectomy
SAS	: Saturated ammonium sulphate solution
SBx	: Surgical bursectomy
SBxSR	: Surgically bursectomised and SRBC inoculated
SBxSt	: Surgically bursectomised and *S. typhimurium* inoculated
SR	: SRBC inoculated
St	: *S. typhimurium* inoculated
SRBC	: Sheep red blood cells
T	: Testosterone
TC	: Testosterone treated, uninoculated
TP	: Testosterone propionate
TSR	: Testosterone treated and SRBC inoculated
TSt	: Testosterone treated and *S.typhimurium* inoculated

Introduction

INTRODUCTION

In India, ducks enjoy second position after chicken as far as their population and egg production are concerned. The total population of ducks during 1972 was 8.43 million, which rose to 14.25 million by 1984, indicating an annual growth rate of 5.75 per cent. Similarly the egg production also increased from 400 million in 1972 to 452 million in 1984 (Sreenivasaiah, 1987).

Duck farming, popular in eastern and southern States of India, has many advantages over chicken farming. They are easy to be reared, produce 40-50 more eggs than chicken per bird per annum and have a longer profitable life. Ducks can withstand extremes of Indian ecological and geoclimatic conditions. They are quite hardy, more easily brooded and can be reared in places such as marshy riverside, wet land and barren moors where chicken or no other type of stock will flourish (Sulbulo, 1983).

The susceptibility pattern of ducks to infectious diseases is quite different from that of chicken and they are generally more resistant to common avian diseases. Lymphatic system is responsible for the normal functioning of immunity. Functionally, the avian lymphatic system is divided into bursa-dependent and thymus-dependent components. The bursal component is held responsible for humoral immunity and the thymic component for cellular immunity (Firth, 1977).

The bursa of Fabricius functions centrally as the progenitor of immunocompetent cells in the humoral immune system, producing antibody molecules - the immunoglobulin. A study on the different classes of immunoglobulins and their characteristics is essential to know their importance in humoral immunity and significance in the pathogenesis of infectious diseases. Though data are available in plenty on the immunoglobulin profile of chicken, similar information is scanty with respect to ducks. Hence an in-depth study of immunoglobulins in ducks is warranted to understand their humoral immune system and resistance mechanisms to various infectious diseases.

Various classes of immunoglobulins identified for chicken are IgG, IgM and IgA. Three subclasses of IgG have also been reported (Higgins, 1975). The concentration and distribution of these immunoglobulins in the sera and tracheal washings of chicken have been studied in detail (Chhabra and Goel, 1980). The immunoglobulins in the egg yolk of chicken have also been studied (Yamamoto et al., 1978). Methods of preparation, purification and concentration of chicken immunoglobulins are also available (Higgins, 1976; Goel et al., 1980; Nandapalan et al., 1983). But not much has been published in this line with respect to ducks, which show quite a different pattern of susceptibility to infectious diseases.

While an intact bursa was found essential during the neonatal period, for the development of humoral immune

competence in chicken (Glick et al., 1956; Chang et al., 1957; Mueller et al., 1962), such an absolute requirement of the bursa in the neonatal period was not found for development of humoral immune responses in ducklings (Glick, 1963). Immunoglobulin profile of duck serum was studied by immunoelectrophoresis and was reported to contain IgA and two sub-classes of IgG, 5.7 S and 7.8 S, differing in sedimentation coefficient (Grey, 1963; 1967a&b; Unanue and Dixon, 1965; Toth and Norcross, 1981a). Hadge and Ambrosius (1984) observed that the 5.7 S IgG did not represent a separate IgG class, but possessed the same 'H' chain of 7.8 S IgG, and suggested that the former lacked the last two homologous constant regions. The biliary immunoglobulins of anseriform birds were found to be IgA-like while those of galliform birds had different antigenic properties in respect to their Fc region determinants (Hadge and Ambrosius, 1980b). Eventhough IgA has been demonstrated in chicken, pigeon and turkey, the characteristics or existence of IgA in ducks are still obscure.

The present study was undertaken with a view to determine the immunoglobulin profile of ducks and to delineate the role of bursa in their production, for which the following techniques were employed:

a) Separation and purification of various classes of immunoglobulins in duck serum and quantitation of immunoglobulins in serum, bile and egg yolk.

b) Quantification of immunoglobulins in ducklings of various age groups which were subjected to bursectomy by surgical, hormonal, chemical or antibursal serum methods, before immunization with either Salmonella typhimurium or sheep red blood cells.

Review of Literature

REVIEW OF LITERATURE

The orientation of the key cells of immunity, the lymphocytes, is effected in the 'training ground' of lymphoid organs, thymus and the bursa of Fabricius or the bursa equivalent.

Bursa of Fabricius

The bursa of Fabricius, endodermal in origin arising as a dorsal diverticulum of the proctodium and developing as early as fourth or fifth day of incubation, is an unique organ present in avian species (Meyer et al., 1959; Romanoff, 1960; Glick, 1963; Ruth et al., 1961; Chakravarthy and Gastry, 1982). The shape of bursa in young adults was round or oval in chicken and cylindrical with oval ends in ducks (Glick, 1963). It measured two to three cm in length and about fifteen mm in width and weighed about three grams in four to five month old fowls (Glick, 1956). The greatest development of the bursa was observed at approximately three to four months in the duck. In the six month old duck, a length of five cm and diameter of seven mm have been recorded (King, 1975). Involution started with the onset of sexual maturity and was completed at the age of ten to twelve months, reducing the bursa to a tiny saccule or fibrous cord embedded in the connective tissue and thereafter totally disappeared (Chakravarthy and Gastry, 1982).

Nagarajan et al. (1980) have reported that the weight

of the bursa of chicken as a percentage of body weight was highest (0.79%) at thirty days of age and then decreased. In White Pekin ducklings, the bursa of Fabricius had a mean weight of 0.08 ± 0.02 g (0.13% of body weight) on the day of hatching and it grew most rapidly during the first five weeks after hatching. The maximum absolute mean of the bursal weight was 2.07 ± 0.48 g (0.09% of the body weight) at nine weeks of age. From the next week there was a decrease in the mean weight, and this was followed by a steady regression. At 22 weeks the weight reached 0.41 ± 0.43 g (0.02% of body weight) (Hashimoto and Sugimura, 1976).

Histology of the bursa

The bursa of the duck has two massive longitudinal folds on the ventral aspect and about 10-12 smaller folds extend into the bursal lumen. These folds contained several lymphoid follicles surrounded by connective tissue trabeculae. Each follicle contained large lymphocytes sparsely distributed in the central region and surrounded peripherally by closely packed small lymphocytes (Chakravarthy and Gastry, 1982). Cellular elements in the follicles were comprised mainly of a series of lymphocytes, epithelial cells and macrophages. In the medulla, which was lined with a sheet of epithelial cells, however, the small lymphocytes were more dominant than in the cortex, which consisted mainly of large and medium-sized lymphocytes. The epithelium coating the inner surface

of the bursa appeared to be divided into two parts, the interfollicular and follicle-associated epithelium. The interfollicular epithelium was pseudo-stratified columnar and the epithelial cells were mucous cells with secretory material. On the other hand, the follicle-associated epithelium was stratified cuboidal or columnar shape which extended into the medulla (Sugimura et al., 1975). The interfollicular space was filled with vascular connective tissue, containing nerve fibres, small number of lymphocytes, plasma cells and granular leucocytes. Plasma cells in this area had already appeared on the day of hatching, and the number increased markedly to the third week of post-hatching life, and thereafter decreased. The number of bursal lymphoid follicles during postnatal life showed almost no changes upto 13 weeks of age. Thereafter a sharp decrease was observed with only a few lymphoid follicles within the bursa at 22 weeks of age, which consisted mainly of hyperplastic smooth muscle and adipose tissue. During natural regression the changes started from 13 weeks of age and were characterized by loss of lymphocytes mainly from the cortex, and thinning of the interfollicular epithelium. Consequently the follicle size diminished. Thinned follicle-associated epithelium gave rise to cystic structures lined by flattened epithelial cells. A marked increase of connective tissue occurred in interfollicular spaces and at 22 weeks, almost all of the bursal lymphoid follicles disappeared and the bursa persisted as a

very small sack near the cloaca for some period after the onset of sexual maturity (Hashimoto and Sugimura, 1976).

Spleen

The splenic primordium appeared at 3.5 days of incubation as a mass of mesenchymal cells. Diffuse lymphoid foci became visible in the spleen immediately after hatching and germinal centres appear by four weeks of age (Thorbecke et al., 1957; DeLanney and Ebert, 1982).

In ducklings, through postnatal life the spleen as a whole varied less in weight than central lymphoid organs such as the thymus and the bursa of Fabricius (Hashimoto and Sugimura, 1976). During the first several weeks of post-hatching life, the spleen grew rapidly and at the third week, its weight as a percentage of the body weight reached its maximum (0.2 %), while the absolute weight showed a successive increase upto the eleventh week (1.36 ± 0.38 g) in spite of a constant decrease of its relative value to the body weight. Beyond this age group, the spleen appeared to reach a stable weight, although a slight decrease in weight was observed from the thirteenth week of age (Hashimoto and Sugimura, 1977).

Periarterioler lymphoid tissue (PALT) and perivenous lymphoid tissue (PVLT) of duck's spleen consisted mainly of small lymphocytes neighbouring the central arteries and collecting or trabecular veins respectively. In aged ducks, germinal centres were occasionally found in these areas.

Periellipsoidal lymphoid tissue (PELT) was the most voluminous in all of the white pulp elements. Splenic distribution of germinal centres occurred in close relation to the vessels exclusively within the PALT or PVLT. They consisted of a variable population of pyroninophilic lymphoid cells and reticular cells suggestive of macrophages. The germinal centres in the PALT were clearly encapsulated with thin reticular fibres, while the ones in the PVLT were occasionally lacking them. The total area of the PALT and the PVLT was less than 10% in the duck spleen, and occupied less than 1/5 of the PELT (Hashimoto and Sugimura, 1977). In the spleen of bursectomised ducks, there was no PELT, but an almost normal number of plasma cell series (Sugimura and Hashimoto, 1976). PELT is considered to be a principal element of the white pulp as well as of the immune response in the duck spleen. The initial germinal centre formation was noted at the seventh week and showed a tendency to increase in number. The germinal centre could not always be found in the spleen of every age group from the seventh week onward, but the frequency of the germinal centres in the PALT was more than ten times that in the PVLT (Hashimoto and Sugimura, 1977).

Among the reticular frame work of the red pulp of duck spleen, cells of the plasma cell line were localized together with the blood cells. This line of cells were frequently detectable near the collecting or the trabecular [redacted]

veins, and occasionally in the venous lumen. The trabecular tissue was less developed (Hashimoto and Sugimura, 1977).

Role of bursa in antibody production

The bursa is the central lymphoid organ from which the immunoglobulin producing cells may originate (Payne, 1971). A review of the literature revealed that most of the studies on the role of bursa in antibody production were in chicken and that only very few such studies were there in ducks.

Glick et al. (1956) found that bursa played a vital role in the production of antibodies to Salmonella typhimurium and that the rapid growth period for the bursa coincided with the period of attaining the ability to develop antibodies to foreign proteins. Once the bird had developed antibody producing ability, it was maintained throughout life. Antibody production was greatly decreased in birds bursectomised during the first few weeks after hatching and the effect declined with increasing age. Bursectomy of young chickens reduced antibody production resulting from injection of S. typhimurium or sheep red blood cells (SRBC). No antibody was demonstrated in sera from chicken bursectomised at two weeks. Those bursectomised at five weeks demonstrated more antibody titre and bursectomy (Bx) at ten weeks produced antibody titre similar to controls (Chang et al., 1957). Intravenous injection of antigen produced higher antibody titres than did intramuscular route. Bursectomised chickens

demonstrated far less resistance to S. typhimurium infection than did the non-bursectomised controls (Chang et al., 1956). The antibody titre following a single injection of antigen was higher at day 7 than at 4, 11 or 18 days (Chang et al., 1957). Sadler and Glick (1961) while studying the antigenacity of duck RBC in bursectomised and non-bursectomised chicken found that 80% of the non-bursectomised birds that were intramuscularly injected with duck RBC had an antibody titre above 1/16 while only 60% of the bursectomised birds had titres above 1/16, thereby indicating the interference of Bx with antibody production.

Glick (1962) reported that Bx did not significantly influence the antibody production of the White Pekin duck. Bx of White Pekin ducklings at four days of age or later, only slightly reduced the antibody response at six weeks of age. At twenty-four weeks, bursectomised birds exhibited a significantly lower antibody titre than controls. This indicated that an intact bursa in neonatal period was not an absolute requirement for development of immune response in ducklings (Glick, 1963).

Removal of the bursa of Fabricius at one day of age effectively prevented a large number of the birds from producing precipitins to bovine serum albumin (BSA) when inoculations were made at 20 weeks of age. But Bx at ten days was less effective. Two steroid hormones, 19-nortestosterone and 17-ethyl-19-nortestosterone, administered on the twelfth

or thirteenth day of incubation, caused a five to twenty-fold reduction in bursal weight and correspondingly, precipitin production against BSA was grossly impaired (Mueller et al., 1962). Fujiwara et al. (1970) found that chicken subjected to Bx and/or thymectomy within 48 hours of hatching and then sensitised with Candida albicans after six or fourteen weeks showed that Bx depressed the development of Candida agglutinins and precipitins, while thymectomy depressed the development of delayed hypersensitivity. Glenbrone et al. (1977) reported that chicks which had been inoculated with infectious bursal disease virus at one day of age had a severe depression of bursa-dependent humoral immune functions by day 42, while it had no significant effect on the thymus-dependent cellular responses. But chicks inoculated with infectious bursal disease virus at twentyone days of age produced near normal antibody responses as compared with the responses in non-infected control chicks.

Glick (1958) found that a crude extract of bursa material increased the antibody titre of bursectomised birds to SRBC. Jankovic and Lechwitz (1968) have reported that neonatally bursectomised chicken were seriously defective in antibody production to human red cells. But when grafted with a bursa in a Millipore chamber, significant restoration of antibody formation occurred. The antibody formed in grafted chicken was ME-sensitive. Chicks bursectomised by testosterone injection on the fifth day of incubation showed

a marked inability to produce antibodies to S. typhimurium. When proteins of the bursa of Fabricius were enclosed in cell impermeable millipore diffusion chambers and implanted subcutaneously or intraperitoneally, the antibody producing capacity of these birds was restored. Evidence strongly suggested that the bursa elaborated a non-cellular agent capable of restoring immunologic reactivity in bursectomised chicks (St. Pierre and Ackerman, 1965). St. Pierre and Ackerman (1966) studied the effect of implants of bursa alone or bursa within cell-impermeable diffusion chambers upon lymphocytic nodules and plasma cells in the spleen of hormonally bursectomised birds immunized with S. typhimurium. Bursectomised birds implanted with empty diffusion chambers failed to produce antibody and lacked lymphocytic nodules and plasma cells in their spleen. Those bursectomised birds with bursa implants produced antibody, had lymphocytic nodules, but lacked plasma cells. Development of lymphocytic nodules in spleen of chicken appeared to be dependent on the humoral action of the bursa, but the humoral agent alone did not appear responsible for plasma cell formation. Studying the effects of injections of bursa of Fabricius extract in normal and bursectomised chicks, Nosakowski and Ziaba (1968) found that antibody production was stimulated in both groups when five injections were given prior to antigen administration. Intravenous transplantation of cells from bursa of Fabricius of six and a half week-old donors to surgically bursectomised

chicks resulted in production of natural and immune antibodies to SRBC and B. abortus (Toivanen et al., 1972a).

St. Pierre and Ackerman (1966) found that implantation of donor bursa in hormonally bursectomised chicken restored the immunological reactivity to S. typhimurium by means of a humoral substance capable of passing through cell-impermeable diffusion chambers. Lymphocytic nodules of spleen, shown to develop under the influence of the humoral agent from the bursa, were thought in part to be the site of antibody production. Heller and Friedman (1979) have reported that the recovery of humoral immune response in bursectomised chicks given crude bursal extracts was of brief duration.

Antibody or antigen was not demonstrated in cells of bursa of Fabricius, by direct fluorescent staining, after immunization of three week-old birds with BSA. But electrophoresis of an extract from the bursa suggested that cells of the bursa may produce Ig, specifically IgM (Glick and Whatley, 1967). The bursa played an active part in antibody formation against inactivated Salmonella culture only upto six weeks after hatching and Bx reduced this antibody formation (Simeonova, 1972). Sato and Glick (1972) determined the antibody titre and class of immunoglobulin in surgically bursectomised birds following one to four immunizations with SRBC. While the primary immune response was lower in bursectomised chicks, there was a normal response to subsequent

antigen injections; following the tertiary injection, IgM synthesis increased in bursectomised chicks. Studying the differential effect of Bx on antibody production in a large and small bursa line of New Hampshire chicken, Landreth and Glick (1973) observed that small bursa line (SBL) birds bursectomised at hatch failed to produce HA to a primary injection of SRBC and Bx at one week reduced the antibody titre. Bx at three and five weeks had no effect on HA levels. In large bursa line (LBL) birds bursectomised at hatch, antibody titre did not differ significantly from controls. Also there were fewer lymphoid follicles at hatch in the bursae from SBL birds than LBL birds.

Removal of bursa of Fabricius at one or five days of age had no significant effect on resistance or development of immunity to fowl pox virus; but chicks bursectomised at fourteen days of age were more susceptible than non-bursectomised birds or those bursectomised at day one. Removal of the bursa at five days did not prevent the development of immunity to New Castle disease virus (Sadler and Edgar, 1958). When seven week-old normal and chemically bursectomised chicken were injected intramuscularly with attenuated Komarov strain of New Castle disease virus (NDV) no difference was found in haemagglutination inhibition (HI) antibody titres of either group.

Separation of the bursa from the rest of the gut associated lymphoid tissue (GALT) showed that the chicken bursa

of Fabricius functioned as a peripheral lymphoid organ since specific antibodies were formed against antigens introduced into the bursal lumen. Evidence was also obtained confirming B. abortus to be a bursa-dependent antigen, but that SRBC needed co-operation of the GALT to evolve a good humoral immune response (Hippelainen et al., 1987).

Bursectomy and Immune Response

Various methods of bursectomy-surgical, hormonal, chemical and using antibursa serum have been tried in birds to study their effect in immune response. Eventhough a lot of research work has been done on the immune response in bursectomised chicken, there are only very few published reports on bursectomy in ducks.

Surgical bursectomy

The removal of the bursa of Fabricius from chicken at ten weeks did not cause a decreased antibody response when the bursectomised birds were challenged with antigen at 22 weeks. However, Bx at one week was associated with greatly reduced antibody responsiveness at six, twelve and twenty-two weeks (Mueller et al., 1960). Eventhough Bx before three days of age did not significantly influence the total of absolute number of leucocytes at 12, 19 or 41 days of age, the small lymphocytes were very few at five and fortyone days than medium sized lymphocytes (Glick and Sato, 1964).

Bursectomy of White Pekin ducklings at four days of age or later slightly reduced the antibody response at six weeks. At 24 weeks, bursectomised birds exhibited a significantly lower antibody titre than controls. This indicated that it may be necessary to allow a certain period of time to pass after Bx of ducklings before an interference in antibody formation can be observed. The longer incubation period of the duck in relation to the chicken may be an important factor in the duck's future antibody maturity. The bursa of the ducks may release its immunologically competent cells or humoral substances during embryonic development. Thus Bx after hatching would be too late to significantly influence the antibody response of the duck. No effect was noted on white blood corpuscles count after Bx (Glick, 1963).

Glaflin et al. (1966) reported that although birds bursectomised (surgically or by 19-nortestosterone) consistently synthesized antibody (chiefly ME-sensitive IgM type), they were immunologically subnormal, as was evident by observing the total antibody titres before and during the first five days after a single immunisation with killed B. abortus. This subnormality was less by day nine and was no longer detected by observing total antibody titres at day thirteen (four days after a second immunisation). This immunological impairment appeared more severe in the case of ME-resistant IgG antibody titres, which were markedly subnormal in bursa-deficient birds at any of the time under study.

Chicks surgically bursectomised at hatching had normal serum IgG levels as late as twelve weeks after hatching and they showed gross IgG deficiency at six months. IgM levels were normal as late as six months after hatching (Jankovic, 1967).

Percy and Bienenstock (1973) reported that Bx at hatching led to pronounced elevation of serum IgM, a moderate decrease in IgG and a more marked fall of serum IgA, when compared to sham bursectomised birds. Van Meter et al. (1969) have reported that despite its suppressive effect on primary antibody responses, Bx at hatching had no effect on the early rise in circulating IgM. This treatment delayed, but did not prevent the normal increase with age of plasma IgG concentration.

The IgG system was quite sensitive to Bx and was more severely affected when earlier Bx was performed, since Bx one week after hatching had no effect on IgG or specific antibody, while Bx at hatching caused a slight lowering in the IgG level and a moderate drop in IgG specific antibody. The IgM system responded quite differently. IgM levels were markedly elevated with earlier Bx, whereas IgM-anti-SRBC titres were little changed. In bursectomised-irradiated birds, both IgG and IgM and antibody levels were low. The fact that the bursa-less bird was able to produce IgM, suggested that the function of the bursa was primarily to

induce the transition from synthesis of IgM to that of IgG rather than to initiate IgM synthesis itself. Under circumstances in which the bursa was prevented at an early time from forming, the potential to produce IgM might develop in another organ, such as the bone marrow, from which the cells which lodge in the bursa were derived. If no bursa formed, the precursor cells might still acquire the potential to produce IgM, but the non-bursal site might not be able to induce efficient differentiation of the IgG system. These results indicated that (1) the bursa had a major role in the maturation from IgM to IgG synthesis and (2) the bursa was not essential for antibody and Ig production (Lerner et al.,1971).

In ovo ex of chicken on days 18, 19 or 20 of incubation and sensitization with SRBC at day 28 after hatching produced undetectably low serum levels of HA, a reduction in SRBC rosette-forming cells with normal or increased 19 S and reduced 7 S immunoglobulins, together with a complete absence of germinal centres in the spleen. The finding that birds which were equipped with serum 19 S but no 7 S Ig lacked splenic germinal centres cast doubt on the hypothesis that the switch of 19 S to 7 S Ig was necessarily an intrabursal event (Blythman and White, 1977). Immunoglobulins made by chicken bursectomised in ovo on day eleven of incubation were studied by two-dimensional gel electrophoresis. All such bursectomised chickens had limited diversity in their Ig molecules. A range of different degrees of diversity

restriction was found in individual bursectomised chicken (Huang and Dreyer, 1978).

Maturation and functional differentiation of immunocompetent cells against B. abortus antigen was observed in surgically bursectomised chicken which had received bursal cell isografts followed by five daily consecutive doses of bursal extract (Baba and Nakahara, 1981).

Cells from chicken bursectomised at 60 hours of incubation and normal controls were examined for Ig and specific antibody secretion in vitro. Anti-tetanus antibodies were observed in the culture supernatants of cells from tetanus-immunised control chicken. But cells from immunised-bursectomised and non-immunised control chicken did not secrete specific antibodies (Darola et al., 1984). Veromaa et al. (1987) reported that the chicken hatched out from embryos surgically bursectomised at 60th hour were unable to respond to antigenic stimulation by specific antibody production. This inability to respond to specific antigens was restricted to B cells and to production of specific antibodies, while the T-cell system was functioning normally. The findings indicated that the bursa was not necessary for the development of thymus-dependent immune functions and supported the suggestion that the specific function of the bursa was the creation of antibody diversity.

Bursectomy at 19th day of incubation caused a decrease

in the number of cells capable of rosette formation and in the amount of antibody produced to SRBC. This was accompanied by an alteration of the response and production of predominantly 2-ME sensitive, high molecular weight antibody (Moticka and Van Alten, 1971).

Losch and Hoffmann-Fezer (1973) conducted histological examination of the bursa, thymus, spleen and caecal tonsils of chicken with abnormal immunoglobulin patterns following embryonal Bx, as well as of their offspring. They suggested that the changes in immunoglobulins might have resulted from secretory disturbances of the plasma cells.

Ivanyi (1975) observed that the antibody responses to four antigens - SRBC, Bordetella pertussis, human serum albumin and influenza virus - were delayed in birds surgically bursectomised at 1, 4 or 7 day after hatching.

Enhancement of antibody response was observed in chicks bursectomised for 5 days after intrabursal priming with SRBC. But Bx performed 2 days after priming resulted in a decreased anti-SRBC response. The enhancement of antibody response was suggested to be mediated by removal of bursa-derived suppressor cells (Hirota et al., 1981).

Surgical bursectomy of chicken at 60 h of incubation, before the appearance of bursal anlage markedly decreased the frequency of IgG^+ cells in the spleen, peripheral blood and thymus. Bursectomy had no effect on the total lymphocyte

and other white cell counts in the peripheral blood (Jalkanen et al., 1983).

Olah et al. (1985) reported that Bdx soon after hatching resulted in cellular depletion of the peri-ellipsoid white pulp and degeneration of ellipsoid associated cells of chicken spleen. They suggested that the impairment of migration of the latter probably accounted, in part, for the small number of germinal centres.

Lerner et al. (1971) postulated that complete suppression of the bursa led to development of bursal cell line precursors in another part of the body. Roticka and Van Alten (1972a) suggested that bursa might not be necessary for all types of antibody responses. Jankovic et al. (1975) suggested by experiments involving Bdx from as early as 52 h of embryonation that the bursa might not be obligatory for the development of the bursal cell line.

Sulochana and Jayaprakasan (1983) reported that chicken surgically bursectomised at third day of hatch failed to respond to a primary vaccination against Newcastle disease virus given on the 8th day, while a revaccination after six weeks produced HI antibodies sufficient enough to resist challenge with a virulent virus.

Quantification of the immunoglobulins in the sera after immunisation with a mixture of antigens in chicken surgically bursectomised at hatching showed that the IgM levels in normal

and surgically bursectomised chicken were essentially the same. In contrast, the increase in the IgG level was hindered until the 6th week in bursectomised chicken. The IgG value in these chicken approximated that in normal ones at about 14 weeks. The influence of SBx varied depending upon the age of the chicken and antigens subsequently injected (Hirota and Ito, 1975).

Nakatani et al. (1986) found a B cell subpopulation in the spleen cells of 3-4 month-old chicken bursectorised on the day of hatching, which had a low density of surface immunoglobulins in comparison with normal B cells.

Hormonal bursectomy

Mueller et al. (1960) observed that prenatal inhibition of bursal differentiation by injection with 19-nortestosterone on the fifth day embryonation (DE) caused greater interference with antibody production than did SBx at one week of age. The data suggested that it was not the overall deficiency in lymphoid tissue in the young adult birds that was responsible for the decreased response to antigen, but the reduced lymphoid tissue in the pre-or neonatal life which had an indirect but long-lasting and critical role in antibody production in later life. Chicken hatched after such treatment were unable to produce precipitins when challenged with a single intravenous injection of bovine serum albumin at six or twentytwo weeks of age. While SBx had no effect on body weight, the hormonal

Bx (HBx) produced birds weighing less than the controls and generally in poor health. Mortality in the hormonally bursectomised birds was also high. Glick and Sadler (1961) found that dipping fertile eggs into solutions containing testosterone propionate (TP) or diethyl stilbestrol caused significant reduction of bursa size and that in some cases TP even eliminated the bursa. Both hormone solutions also reduced spleen size. The antibody response to a polyvalent Salmonella pullorum antigen was markedly reduced in birds hatched from the TP dipped eggs.

Testosterone administered on 12th or 13th D2 caused a five to twenty-fold reduction in bursal weight (Mueller et al. 1962) and significant reduction of bursal size was also seen when given to eight week-old Rhode Island Red birds (Glick, 1957). Treatment of White Leghorn chicks with low doses of TP (0.625 mg/chick daily) for the first two weeks of life greatly reduced the bursal weight without influencing the weight of thymus. The relative weight of spleen was reduced on the 30th and 44th days of life only (Rosolowska-Huszcz and Skwarlo, 1980). A quantitative reduction in bursa size at hatching also occurred after dipping eggs in TP solutions of 320 mg/100 ml ethyl alcohol or higher concentrations. A reduction in antibody response also occurred in birds hatched from the TP dipped eggs (May and Glick, 1964). Rose and Orlans (1969) found that Hbx with testosterone was more

effective than SBx coupled with irradiation in depressing the primary response to soluble antigens.

Testosterone propionate dipping (TPD) method was found to be more efficient in effecting embryonic Bx than the TP injecting (TPI) method, as the majority of late embryos and day-old chicks from TPD-treated eggs lacked a visible bursa (Glick, 1961; Glick and Sadler, 1961), while the bursa was present in those injected with TP on day 12 of incubation (Glick and McDuffie, 1974; Warner et al., 1969). Glick and McDuffie (1974) also observed that during the first week of embryonic life, the bursa alone was steroid-sensitive, while during the second week both bursa and stem cell may be steroid sensitive.

Lerner et al. (1971) observed that Bx performed as early as the 3 DE with testosterone resulted in marked lowering of IgG. The IgM levels were markedly elevated with earlier Bx, whereas IgM anti-SRBC titres were little changed. Hoffmann Fezer and Losch (1973) observed the following Ig patterns in fowls that were hormonally bursectomised after 3 DE and observed between ten weeks and 18 months: IgG levels were either low with normal or increased IgM concentrations or vice versa and both IgM and IgG levels were either low or normal. Hirota and Ito (1975) produced hormonally bursectomised chicken by either dipping 3 DE eggs in 2% TP in ethanol (TP_3), or by inoculation into chorioallantois

2.66% TP in corn oil in 12 DE embryos (TP_{12}). The antibodies produced by TP-treated chicken against SRBC and Salmonella pullorum were almost exclusively IgM type. Treatment with TP on 3 DE resulted in much higher IgM levels than in normal birds, but the treatment on the 12 DE suppressed it to some extent. IgG production by the TP_3 chicken completely stopped until 6 to 7 weeks of age, while that by the TP_{12} chicken was suppressed considerably until about 10 weeks of age, after which it started to increase rapidly. Normonally bursectomised chicken responded to SRBC to a higher extent than those surgically bursectomised in a newly hatched period. Hirota et al. (1976) also reported that the production of IgM antibody against SRBC was not affected significantly by TP, but immune responses against bacterial antigens and the production of IgG antibodies were strongly suppressed.

Injection of TP into 12 DE eggs prevented the development of lymphoid tissue in the bursa of Fabricius in chicken. When immunological responses of these hormonally-treated chicken against certain antigens were studied no circulating antibody was detected (Warner et al., 1962). Carey and Warner (1964) observed that the chicken hatching out from embryos treated at 12 DE with 4 mg TP and subsequently immunized with bovine serum albumin at four weeks produced less than one percent of normal levels of antibodies, but synthesized at least 40% of the normal amount of gammaglobulin. On rechallenging several of the TP_{x} chicken that survived

until eight months with various antigens, a total lack of antibody production to most antigens was found, eventhough some of these birds had considerable amounts of IgG and/or IgM. These results reinforced that concept of an absolute dissociation of immunologic responsiveness in chicken, in that the bursa was the sole site of control of the development of the antibody forming system (Warner et al., 1969).

Glick (1969) reported that Bx using alcoholic TP solution did not significantly influence the absolute number of lymphocytes, and heterophils or the haematocrit and haemoglobin values. Chicks were injected intramuscularly with 2.5 or 7.5 mg TP twice daily for the first four days after hatching. The 2.5 or 7.5 mg dosages reduced bursa weight at 19 and 43 days of age, respectively. At 19 days of age, the bursa was almost completely devoid of follicles, while at 43 days there were numerous enlarged bursal follicles. The number of bursal dependent follicles in the spleen was reduced before 43 days of age. The antibody response to both SRBC and bovine serum albumin was less than that of controls at four to five weeks. When the immunization was delayed until nine or eleven weeks, the antibody response was normal. The delay in normal antibody response might be by the control exerted by the bursa over splenic development (Glick, 1970).

Chicks from eggs treated with TP on twelfth day of embryonation produced almost entirely IgM antibodies to SRBC and influenza virus, and no IgG responses to Brucella and

Salmonella. The IgM antibody response to SRBC by spleen cells of treated chicks was higher than by cells from normal birds. The unusual B cell fractions in treated birds were considered to be those of a distinct B cell subpopulation having immune responses restricted to the IgM type and the development of which was independent of the bursa of Fabricius (Hirota et al., 1979; 1980).

Lupetti et al. (1983) observed that daily intramuscular administration of 5 mg TP for four days from hatching resulted in a decrease of bursal weight.

Eventhough the reports on Bbx in chicken are numerous, only very few such studies have been conducted in ducks and other birds. Olick (1963) reported that while the antibody response of the White Pekin duck was not influenced by Bbx at hatching, dipping fertile duck eggs into 2 g % TP on fifth day of incubation significantly reduced the bursal size and these treated ducks failed to respond to Salmonella pullorum. No significant differences were found for day-old body weight and spleen weights of ducklings hatched from eggs dipped in 670 mg (TP_{670}) or 2 g (TP_2) of TP per 100 ml of ethyl alcohol. A significant body weight reduction was recorded at four weeks of age for the TP_2 treated ducklings, while the TP_{670} and control ducklings exhibited no body weight differences. The antibody response of the 6-week old TP_2 ducklings to SRBC was eliminated, while that of the TP_{670} ducklings was the same as the control group. At 6 weeks of age, the bursa was absent

in the TP_2 ducklings, while it was present but reduced in size in the TP_{670} group. Histological sections of day old bursae from the TP_2 birds demonstrated an absence of lymphocytes, while lymphocytes were present in the bursae of TP_{670} and control ducklings.

Chemical Bursectomy

The suppression of humoral immunity but not cell-mediated immunity with cyclophosphamide (Cy) was first demonstrated in the chicken by Lerman and Weidanz (1970). Treatment of chicks with 4 or 6 mg of Cy for each of the first three days of life suppressed the primary and secondary responses to bovine serum albumin (BSA), sheep red blood cells (SRBC) and Salmonella typhimurium, and also reduced the levels of IgG and IgM. The IgM and IgG levels in the serum of some of the Cy-treated birds were less than 0.5 per cent of the Ig levels found in untreated birds of the same age. Glick (1971) observed that the usual basophilic lymphoid cells of the bursa were replaced by large, pale reticular cells in 2-day-old chicks treated on the previous two days with 6 mg of Cy per day. No regeneration of the bursa was observed in these chicks, but chicks receiving a single injection of Cy showed regeneration of some bursal follicles.

Kirchner et al. (1972) have reported that Cy treatment of chicken during early neonatal period selectively inactivated B cells as indicated by a gammaglobulinemia. Cy given

on days 1, 2,and 4 post-hatching was found to cause lymphocytic depletion of the bursa, thymus and spleen. The toxicity of Cy varied with the breed and strain of chicken and some birds even regained their immunocompetence (Rouse and Szenberg, 1974).

Cyclophosphamide has been demonstrated to remove the lymphoid element from embryonic bursae without destroying the stroma (Eskola and Toivanen, 1974). Testosterone destroyed the capacity of the bursa to serve as a differentiation site for the B-cell lineage, affecting the stromal cells of the bursa. But Cy destroyed only the lymphoid population undergoing differentiation, leaving the bursal stroma intact. A long lasting and severe humoral immuno-deficiency occurred on intravenous administration of Cy to chick embryos on days 14-16 or 16-18 of incubation (Eskola and Toivanen, 1974). At 44 days after hatching, only rudimentary follicles and increased interfollicular connective tissue were found in the bursa of Fabricius. Hirota and Bito (1978) reported that neonatal treatment of chicks with Cy and X-ray irradiation suppressed completely or almost completely antibody responses, Ig production and formation of bursal follicles and splenic germinal centres.

While investigating the effect of Cy on the thymus and the bursa of Fabricius in chicken of one day to seven weeks of age, Hiraga et al. (1976) found that the relative weights

of both the thymus and the bursa decreased abruptly just after the injection of Cy and never returned to the control level until 7 weeks of age. Degeneration of lymphocytes, an increase of macrophages, hypertrophy of the reticular cells, and a disappearance of mitotic cells were observed in the cortex during early involution. Restoration of the lymphoid follicles was observed from 5 to 10 days of age, mainly in the groups treated with a smaller quantity of Cy. In the chicken, treated with 18 mg Cy, however, only a few restorative follicles were found. Differences in sensitivity to Cy action was also observed among the lymphocytes, depending upon their size and location.

Fiedler et al. (1977) observed that neonatal Cy treatment at a dose of 8 mg/bird resulted in incomplete immunosuppression, with a tendency towards recovery from the seventh week. But a relatively small dose of Cy combined with neonatal Bx produced complete suppression of the humoral immune system over a long period. Cy-treatment combined with Bx eliminated the B-cell compartment in the bursa, spleen and caecal tonsils upto 13 weeks of age and no recovery occurred (Hoffman-Fezer et al., 1977).

Prasad (1978) observed that administration of 4 mg of Cy/chick for three consecutive days post-hatching caused depletion of lymphoid cells in bursa, thymus and spleen. The bursa and thymic lobes were reduced two to three times

but the damage to both the bursa and thymus-dependent immune mechanisms produced by Cy was reversible in five weeks time. Sachs et al. (1979) recorded that Cy decreased bursal weight and the agglutinin titre response to SRBC. Histological changes of bursa included decreased plical size, thinning of the follicle-associated epithelium, hyperplasia of the inter-follicular epithelium, decrease in the number of medullary lymphocytes and virtual absence of cortical lymphocytes. Baba et al. (1982) examined histologically the thymus and bursa of chicks, killed at intervals (1-28 days), after intra-peritoneal injection of 3 mg/100 g body weight of Cy daily for 1-3 days. Follicular atrophy and cellular depletion were seen 24 h after the third injection and decrease in bursal folds and thymic lobules after 48 h; regeneration occurred from 10 days and was almost complete by 23 days.

Sugimura et al. (1975) suggested that Cy was better suited for Bx of ducks than testosterone, since testosterone produced extremely high mortality in the prehatching period. The total dosage of Cy required in ducks was smaller than that employed in chicken, which made effective chemical Bx in ducks possible (Hashimoto and Sugimura, 1976). Cy-treatment of post-hatching ducklings resulted in destruction of lymphoid cells of the bursa and replacement of the follicle-associated epithelium by a mucous one (Sugimura et al., 1974; Sugimura and Hashimoto, 1976). In newly hatched White Pekin

ducklings. Cy-treatment produced significant reduction in the weight of their lymphoid organs and almost completely eliminated the formation of bursal lymphoid follicles at one week of age. Eventhough the thymus and spleen of these ducks completely recovered in weight at seven weeks of age, the bursa remained as such (Hashimoto and Sugimura, 1976).

One of the most apparent diminutions of cellular components of blood appeared in the leucocytes and thrombocytes after Cy treatment. These changes completely recovered at seven weeks of age, and the absolute number of lymphocytes was predominant over that of the control ducks.

Electrophoretic analysis of serum revealed that at seven weeks, the controls showed a double-peaked pattern at the beta-globulin corresponding site. However, the serum from treated ducks indicated not only a blotting out of the beta peak, but also a decrease in the gamma-globulin level (Hashimoto and Sugimura, 1976).

Study of the effects of Cy on natural antibody levels in chicken showed that the drug suppressed agglutinin titres in newly hatched chicks for upto twelve weeks of age. When given at seven days, the suppression was found upto eight weeks, but titres rose to normal by ten weeks after initial injection. When given after 21 days of age, the drug did not suppress agglutinin titres unless it was administered daily for two or more weeks (Dutinger and Hirata, 1972).

Glick (1966) found that air cell application of Cy between 16-18 DE was an effective route for the abrogation of bursal lymphoid development and suppression of humoral immunity. The bursae from the Cy-treated birds were significantly reduced in size, deficient in bursal follicles, and lacked lymphocytes. The agglutinin level of SRBC of birds treated with 2 mg Cy as 16, 17 and 18 day embryos was significantly lower than controls. While these Cy-treated birds lacked IgG antibody to SRBC, about 50% of the Cy birds produced unspecific IgG. Ouchterlony analysis evinced the presence of IgM in 60% of the IgG negative birds. Cy did not change the absolute number of lymphocytes, granulocytes or erythroid series of cells in the bone marrow, but did eliminate plasma cells. Since some of the Cy-treated birds did not produce specific agglutinin but made Ig, it was concluded that the presence of the bursa was not obligatory for Ig synthesis, but that the bursal microenvironment might be a prerequisite for synthesis of specific antibody.

Use of antibursal serum in bursectomy

Bursectomy and thymectomy have been supplemented by the use of antibursal serum (ABS) or anti-thymus serum (ATS). The advantages of these antisera are selective killing of cell types regardless of their organ localization, ease of treatment, and feasibility of *in ovo* selective destruction of cell types (Kramer, 1975). Wick *et al.* (1973) also

emphasized the advantage of avian antilymphocyte serum studies in chicken. They assayed the serological properties of turkey antiserum to chicken bursa and thymus cells. In complement-dependent cytotoxicity tests, ABS and ATS were found to react specifically with bursa and thymus cells respectively. In the spleen of two and three week-old chicken, 20-30% of lymphoid cells were killed by ABS and 40-50% by ATS. Fesce et al. (1970) observed that ABS and ATG, prepared in rabbits, showed similar activity against suspensions of both organs in vitro. Both had a similar immunosuppressive effect in chicks previously immunized against Newcastle disease, as demonstrated by haemagglutination tests before and after the injection of antiserum. They suggested that the antilymphocytic serum acted as a competitive antigen. Daily injection of antithymus globulin (ATG) or antibursa globulin (ABG) into the chorioallantoic space of chick embryos from the seventh to seventeenth days of incubation revealed that ATG affected the cytology of both spleen and bursa, while ABG affected only the bursa (Jankovic et al., 1970). Both ATG and ABG depressed antibody production in four week-old chicks repeatedly inoculated intraperitoneally with ATG/ABG and simultaneously inoculated with bovine gamma globulin. In the spleen, ATG depleted lymphocytes while ABG depleted plasma cells. Both preparations and normal rabbit globulin produced a fall in the lymphocyte and granulocyte counts of peripheral blood.

Prasad (1978) made a study of the changes in the lymphoid organs of chicken treated with rabbit antichick thymocyte serum (ALS) and their correlation with the cellular immune function. The results indicated that the immunosuppressive effect of ALS was independent of lymphocyte depletion in lymphoid organs.

Sawada and Hito (1980) transferred chicken B cells treated with diluted antibursa cell serum in the presence of complement together with normal T cells and SRBC, into immunodeficient chicken. Spleen cells taken from these were examined for plaque-forming cells. They found that the production of IgM plaque-forming cells was considerably more resistant to the cytotoxic effect of the antiserum than that of IgG plaque-forming cells. Such differential susceptibility of development of IgM and IgG plaque-forming cells was observed only in chicken aged about two weeks or younger. Similar results were also obtained by Nakatani et al. (1986) who treated spleen cells from neonatally bursectomised chicken with various dilutions of antibursa cell serum in the presence of complement and carried out tests on their immunocompetence.

Baba et al. (1983) prepared antisera to bursal extracts or perfusates and investigated the influence of such sera on antibody production in chicken by the injection of antisera during the embryonic stage. Antisera to Cy-treated bursal

extracts or bursal perfusates were injected on the 15th day of embryogenesis. The level of antibodies produced by chicken treated by these antisera was found to be equal to the controls but IgG antibodies were totally absent. At the same time, hormonally bursectomised chicken that were administered lymphocyte related substance free bursa extract from Cy-treated chicken, restored the IgG antibody production. This indicated that bursa lymphocyte related substances were not responsible for the switch over to IgG. These results showed that the bursa specific antibody contained in the administered antiserum caused damage to some bursa tissues other than bursa lymphocytes, resulting in the disturbance of secretion of bursal humoral factor which induced conversion of IgM producing cells to IgG producing cells.

Role of bursa in development of immunological competence

Two theories have been put forward concerning the role of the bursa in the development of immunological competence - (i) The bursa is a source of immunologically-competent cells which are seeded out to other tissues, (ii) the bursa secretes a hormone necessary for the development of immunocompetence by the immunoglobulin synthesizing system of cells of non-bursal origin (Payne, 1971).

Work carried out by various scientists illustrated the importance of the bursa as a microenvironment in which differentiation and maturation of immuno-competent cells take

place. Cy has been demonstrated to remove the lymphoid element from embryonated bursae without destroying the stroma (Eskola and Toivanen, 1974). Using cell-transfer techniques into Cy-treated birds, Toivanen et al. (1972a) concluded that only bursal cells were capable of morphological and functional reconstitution of the bursa-dependent lymphoid system. They divided the bursal cells into 2 basic types - (i) bursal stem cells - which were capable of restoring normal bursal morphology and required the bursal microenvironment for maturation. They were also capable of restoring germinal centres in the spleen, and were found in the bursa during the first few weeks after hatching. (ii) post-bursal cells, which could not restore bursal structure and were independent of the bursa for further maturation. They were divisible into early and late types by the ability of the former to multiply in treated bursae and to readily form germinal centres. The late post-bursal cell has no clear effect on germinal centres and is the principal cell in the bursa and bone marrow after the 10th week of life. Toivanen et al. (1972b) found that the development of immunocompetent cells followed the development of the bursal stem cell population. The post-embryonic stem cell responsible for humoral immunity emigrated from the bursa to the bone marrow at the time of bursal involution. Subsequently a cell with the same reconstituting capacities appeared in the spleen and to some extent also the thymus (Toivanen et al., 1972c).

The bursa seeded out immunocompetent cells during its entire post-embryonic development, but did not release the lymphoid stem cell population before this population has matured sufficiently and before the bursa itself started to involute (Toivanen et al., 1973b). Cooper et al. (1974) reported that lymphocytes in the embryonic bursa appeared in sequence of IgM, IgG and IgA cells. An intraburseal switch mechanism was thought to differentiate lymphocytes in such a cycle. Once out of the bursa the lymphocytes were committed and independent of the bursa.

Glick (1960) and Cooper et al. (1966) favoured the role of a bursal hormone in the development of immunocompetence. Gilmour et al. (1977) isolated and purified the bursal hormone (Bursopoietin) which had higher specificity for B cell induction than the earlier crude extracts. Audhya et al. (1986) reported that bursin, a selective B-cell differentiating hormone of the bursa, isolated from the bursa Fabricii of fowls induced the phenotypic differentiation of mammalian and avian B precursor cells but not of T precursor cells in vitro.

Hashimoto and Sugimura (1980) studied the distribution and morphology of antibody-producing cells in White Pekin ducks by light and electron microscopic immunocytology. The typical antibody producing cells were the plasma cells and large lymphoid cells in splenic red pulp or germinal centres.

In the germinal centre region, two types of positive cells were identified, the predominant positive cells in quantity were dendritic cells, and the other was typical antibody producing cells. The results suggested that some of the antibody producing cells of ducks originated in the germinal centres during the course of response, and germinal centres of duck spleens and lymphnodes functioned as an indispensable lymphatic element in their antibody-producing system. Higgins and Chung (1986) suggested that since ducks are phylogenetically close to the amphibians and reptiles, their lymphocyte population may not express surface markers similar to those of chicken and mammals.

Immunoglobulin synthesis by bursa cells

During maturation of the egg, maternal antibodies are secreted into the yolk sac from the secretory follicles in the epithelial lining of the oviduct. The predominant Ig of yolk is IgG. Yolk antibodies were transmitted in increasing amounts from 11 days of incubation to hatching (Leslie, 1975). At 15 days of embryonation, no IgM or IgA was detectable in the serum of embryos and the IgG level is only 2-4% of that seen at hatch (Leslie and Martin, 1973). Thus most of the IgG was absorbed into the embryonic circulations during the final 5-6 days of embryonation. IgM is usually undetectable in chick serum until 3-4 days after hatch. With *in vitro* culture techniques, 16-18 day embryonic bursas have

been shown to synthesize IgM (Leslie, 1975). Using a fluorescent antibody assay, Kincade and Cooper (1971) demonstrated IgM in the bursal lymphoid cells of 14-day old embryos. The ontogeny of serum IgG was complicated by the presence of yolk derived IgG. But chicks raised from hen with severe IgG hypogammaglobulinemia have shown serum IgG within 3-6 days after hatch. IgA was undetectable in the serum until about 12 days after hatching. Peppard et al. (1983) have provided evidence for the existence of a molecule in chicken homologous to the secretory component of mammalian IgA. The presence of IgA has not so far been detected in ducks.

The dependency on the bursa for the switch from IgM to IgG was established by a series of experiments. In ovo bursectomies performed early (17 DE) or late (19-21 DE) impaired IgG synthesis (Van Alten et al., 1968; Cooper et al., 1969; Meticka and Van Alten, 1971, 1972a,b).

Bursa-independent immune system

Production of antibody in hormonally bursectomised (TPX and TPS) birds in which embryonic bursal development was abolished (Glick and Sadler, 1961; Claflin et al., 1966; Glick, 1968) led to the formulation of the theory that 'other sites in chicken were capable of conditioning or supplying immunocompetent cells (Glick, 1968; Lerner et al., 1971). In ovo Bx at 72 hours eliminated the bursa and one-third of large intestine in the hatched chick. These birds produced

agglutinins to SRBC, and six out of eight birds made Ig (possibly IgM) (Fitzsimmons et al., 1973). Surgical removal of the caudal portion of the tail bud before 64 hours of embryonic development did not eliminate lymphopoiesis of cells possessing bursal membrane determinants (Jankovic et al., 1975). These bursal cells were evident in spleen, bone marrow, and thymus of 21 day-old bursaless embryos. This led Jankovic et al. (1975) to postulate that the chicken had two antibody producing systems, one bursa-dependent and the other bursa-independent.

Lerner et al. (1971) suggested that the bursa functioned as a microenvironment for inducing the transition from IgM production to IgG or IgA production, and that another lymphoid tissue, such as the bone marrow was available in bursaless chick.

Comparative studies of B-cell development in the bursa and bone marrow of the chicken after hatching provided support for the view that bone marrow played an important role in the generation of the B-cell repertoire (Kincade et al., 1973). Moticka (1975) also postulated that the bone marrow could be a non-bursal site for B-cell differentiation. Glick and Rosse (1981) suggested that avian bone marrow might possess a progenitor pool for virgin B cells that was distinct from B cell progenitors in the bursa and was independent of that organ. Befus et al. (1980) on the

other hand postulated that the widespread mucosal network comprising of the bronchial lymphoid aggregates, Harderian gland and gut associated lymphoid tissue may in addition to acting as secondary lymphoid tissue, represent the bursal independent sites of B cell differentiation in chicken.

The non-bursal environment may function only in the absence of the bursal environment. Hence the early removal of the bursa by Bx leads to activation of potential sites for B-cell differentiation.

Avian Immunoglobulins

Among the avian immunoglobulins, fowl immunoglobulins have been studied in great detail. Information on immunoglobulin of other avian species are limited.

Chicken have been known to produce at least three major classes of immunoglobulins - IgG, IgM and IgA (Higgins, 1975). Goel (1984) observed an Ig-like component in chicken serum, the beta component, with a molecular weight of 190,000.

The structure and properties of fowl IgM resemble, in general, those of IgM of other species. The average molecular weight is 890,000 and structurally IgM occurs as a pentamer. Schranner and Losch (1986) have identified in chicken serum, the monomeric state of IgM (molecular weight 184,000) along with the pentameric state (molecular weight 920,000). The mean serum levels of IgM in adult fowls of unspecified age and sex have been stated to be 0.71 $\pm$ 0.18 mg/ml with a

range of 0.5 to 0.93 mg/ml (Leslie and Clem, 1970), and as 1.25 mg/ml (Leslie and Martin, 1973). Fowl IgM can be reduced with 2-mercaptoethanol, cysteine or dithioerythritol. With mild reduction the IgM polymer is reduced to its monomeric form and antibody activities requiring multiple valency are suppressed (Benedict et al., 1963b,c; Rosenquist and Campbell, 1966; Leslie and Benedict, 1968; Kuniyasu, 1969).

The IgG of chicken differs physicochemically from mammalian IgG in that it has a larger molecular weight, more carbohydrate and displayed an unusual heavy-light chain interaction. This molecule, as in the mammal, is the major serum Ig and has been called IgY by some workers (Leslie and Clem, 1969). IgG is easily reduced and its antibody activities, including precipitation can be diminished or eliminated by mild reduction with 2-mercaptoethanol or dithioerythritol (Benedict et al., 1963a; Szendberg et al., 1965). Serum levels of IgG in adult birds have been found to be 3.29 ± 1.35 mg/ml, with a range of 4.1-7.3 mg/ml (Leslie and Clem, 1970). At 6, 7, 8 and 10 to 12 weeks, levels of 3.0, 3.8, 12.6 and 12.5 mg/ml were recorded (Warner et al., 1969), and at 44 days, IgG levels were 2.7 mg/ml with a range of 0.3-4.2 mg/ml (Cooper et al., 1969). Three possible subclasses of fowl IgG, G_1, G_2 and G_3, have been identified (Watanabe and Isoyama, 1973).

Lebacq-Verheyden et al. (1972) first demonstrated an

Ig in chicken which was not IgM or IgG and which predominated in secretions, and provisionally called it 'IgA'. While not all the properties of this fowl secretory Ig were the same as those of mammalian IgA, there are similarities in the physical and chemical structure and function (Higgins, 1975). Schranner and Losch (1985) observed that serum IgA existed in dimeric (340,000 d) and in monomeric (170,000 d) states. In the serum of adult fowls the level of IgA is 0.61 mg/ml (Leslie and Martin, 1973). Watanabe et al. (1975) identified a homologue of a free secretory component in chicken intestinal secretion.

The three classes of immunoglobulins - IgG, IgM and IgA, have been isolated from turkey and pigeon. Saif and Dohms (1974) isolated IgG and IgM from turkey serum. Turkey IgA was isolated from bile, intestinal secretion and serum (Lim and Maheswaran, 1977), and also from saliva, lacrimal secretions and tracheal washings (Dohms et al., 1978). Goudswaard et al. (1977) isolated IgG and IgM from pigeon serum and IgA from bile. Pigeon IgA was also isolated from crop milk and IgG from egg yolk (Goudswaard et al., 1979).

Grey (1967a,b) found three immunoglobulins in ducks, IgM and two low molecular immunoglobulins with sedimentation co-efficients of 7.8 S and 5.7 S. The IgM had antigenic and structural properties similar to that of mammalian IgM (Hadge and Ambrosius, 1984). Pamela and Higgins (1986) found

that duck serum IgM had a molecular weight of 800,000 daltons and 'H' chain of 86,000 daltons. High cross-reactivity has been observed between chicken IgG and duck 7.8 S Ig (Zimmerman et al., 1971; Hadge and Ambrosius, 1984).

Studies by Zimmerman et al. (1971) revealed that duck gamma-heavy chains were held together by a higher number of disulphide bonds than were the mammalian gamma-H chains, thereby making the duck gamma-H chain extremely rigid and inefficient in reactions such as precipitation and agglutination. Toth and Norcross (1981b) observed that duck immunoglobulins appeared to be inherently deficient in immunological reactions like precipitation and agglutination which required functional bivalency.

Grey (1967a) found that in normal Muscovy ducks, the 7.8 S Ig was the major Ig, representing 70 to 80% of the low molecular weight Ig. In normal Pekin and Mallard ducks, however, the 5.7 S and 7.8 S proteins were present in roughly equal quantities. Following hyperimmunization, all ducks showed a relative increase in the 5.7 S Ig.

The characteristics or existance of duck IgA are still obscure. Eventhough Grey (1963) designated a duck serum protein as IgA on the basis of its electrophoretic characteristics, it was identified as a minor duck IgG (Toth and Norcross, 1981a). Parry and Aitken (1975) also failed to detect any cross-reacting homologous protein in sera or

secretions (Saliva and bile) of ducks on doing immunodiffusion tests using rabbit and pheasant antisera monospecific for fowl IgA. At the same time, homologous antigens were detected in guinea fowl, quail, turkey and pigeon.

Immunoglobulins in bile and egg yolk

Watanabe and Kobayashi (1974) purified IgA from chicken bile and found that it resembled the serum type of high polymeric IgA, lacking a secretory component and having a molecular weight of 800,000 to 900,000. The existence of a functional homologue of mammalian secretory component in chicken bile IgA was established by Peppard et al. (1983, 1986). Sanders and Case (1977) reported that IgA was the only Ig present in fowl bile. But Mockett (1985) demonstrated IgM in chicken bile, for the first time, using an immunoadsorbent prepared from monoclonal antibody for IgM.

Pamela and Higgins (1986) found Ig of a single class in duck bile, with a molecular weight of 890,000 and 'H' chain molecular weight of 75,000. Antigenic comparison showed that bile Ig resembled IgM, but carried additional determinants. They suggested that duck biliary Ig was an IgM-like molecule secreted independently of serum Ig. Studies by Hodge and Ambrosius (1988b) on the antigenic properties of the biliary immunoglobulins of galliform (chicken and turkey) and anseriform (ducks and geese) birds revealed that the biliary immunoglobulins of anseriform birds

were IgM-like while those of galliform birds had different antigenic properties in respect to their Fc region determinants. In a comparative study on the structure of biliary immunoglobulins from chicken, turkey, duck and goose, Hadge and Ambrosius (1988a) observed that the bile from these birds contained immunoglobulins in relatively high amounts of 4.5 to 15 mg/ml.

Data on the immunoglobulins of egg yolk are available mainly for fowl (Patterson et al., 1962; Wilkinson and French, 1969; Kramer and Cho, 1970; Watanabe and Isayama, 1973; Yamamoto et al., 1975), turkey (Saif and Dohms, 1974; Goudswaard et al., 1977) and pigeon (Goudswaard et al., 1979). Most of these research workers are of the opinion that egg yolk contains only IgG. But Yamamoto et al. (1975) observed IgM and IgA also, besides IgG, in concentrated preparations of egg yolk of chicken. The quantification of IgG in egg yolk has been attempted only in the domestic fowl and in the turkey. The amounts of IgG in yolk was reported to be 20-25 mg/ml in hen's egg and 2-6 mg/ml in turkey's egg (Rose and Orlans, 1981).

Separation and purification of serum globulins

Various techniques have been evolved to separate and purify serum immunoglobulins, based on their differences in physico-chemical properties. Prior to the successful separation of any specific Ig, a preliminary fractionation

procedure is usually employed to present it in high yield and concentration.

Fractionation with neutral salts

Fractionation with neutral salts like sodium sulphate or ammonium sulphate have given good yields of Ig. Benedict (1967) observed that immunoglobulins of chicken can be precipitated from serum at room temperature, by the addition of 18%, 14% and 14% of crystalline sodium sulphate in three successive steps. Later various workers (Higgins, 1976; Goel et al., 1980; Nandapalan et al., 1983) have precipitated chicken globulins and Saif and Dohms (1976) precipitated turkey globulins, using this technique. Globulins of ducks were fractionated using sodium sulphate at two successive concentrations of 50% and 33% respectively (Toth and Nororcss, 1981a).

Weir (1967) precipitated gamma globulins of many mammalian sera by adding saturated ammonium sulphate solution to the serum to a final concentration of 33 per cent. Herbert (1974) used three precipitations in 35 per cent saturated ammonium sulphate for fractionation of chicken serum globulins. Fei et al. (1986) precipitated duck serum globulins using 40 per cent saturated ammonium sulphate.

Chromatography of serum immunoglobuling

After preliminary fractionation procedure, the globulins obtained may be purified by chromatographic techniques. Three

types of such techniques have been used to purify avian immunoglobulins - gel filtration using sephadex G-200, ion-exchange chromatography using DEAE cellulose and affinity chromatography using immunoadsorbants.

Gel filtration chromatography

In gel filtration chromatography, gels of cross-linked dextran, agar, agarose or polyacrylamide beads are used for the separation of sucstances of different molecular dimensions. The principle of gel filtration is that it utilises the molecular sieve properties of gels.

Gel filtration using sephadex G-200 has been employed in the purification of chicken immunoglobulins. Fractionation of chicken serum proteins by this method resulted in the elution of proteins in two main peaks, the first major peak being largely composed of IgM and alpha-2 macroglobulin and the second major peak composed of IgG (Higgins, 1976; Goel et al., 1980; Nandapalan et al., 1983). Higgins (1976) used 0.1 M tris-HCl buffer, pH 8.0, containing 1 M NaCl and 1 m M ethylene diamine tetraacetate (EDTA) for gel filtration through sephadex G-200, while borate buffered saline, pH 8.2, was used as sephadex buffer by Goel et al. (1980). Turkey IgM and IgG were also fractionated using sephadex G-200 (Saif and Dohms, 1976; Lim and Maheswaran, 1977).

Grey (1967a) further separated the duck gamma globulin

fractions obtained by preparative starch block electrophoresis, using sephadex G-200 gel filtration in a 2.5 x 100 cm column with a buffer composed of 1 M NaCl and 0.1 M tris, pH 8. Then three elution peaks were observed, the first corresponding to the gamma M fraction along with varying amounts of lipid and aggregated material. Two incompletely resolved peaks followed the first peak, representing the 7.8 S and 5.7 S globulin fractions respectively. Grey (1967a) also observed that the relative heights of the second and third peaks varied with the species of duck and state of immunization. Normal Muscovy ducks always had a predominant second peak and a rather minor third peak, whereas normal Pekin and Mallard ducks had second and third peaks of roughly equal height. Upon hyperimmunization, all ducks demonstrated a major third peak and a minor second peak. When individual peaks were pooled, concentrated and rerun on the same column, purified immunoglobulins were obtained. Toth and Norcross (1981c) fractionated pooled serum samples from ducks on a 2.5 x 90 cm Sephadex G-200 column with 0.05 Tris buffer (pH 8) containing 0.001 M EDTA to obtain IgA and IgG. Fei et al. (1986) chromatographed ammonium sulphate precipitated duck globulins through Sephacryl S-300 column to obtain 7 S and 8 S globulins.

Ion exchange chromatography

Ion exchange chromatography has been proved effective

for fractionation of antibodies and purification of immunoglobulins. In this technique, an insoluble adsorbent is commonly packed into a column, and buffer conditions are adjusted so that adsorbent and soluble proteins have opposite charges. Proteins become fixed to the adsorbent through electrostatic bonds and they may be eluted sequentially by raising the ionic strength or/and by changing the pH of the buffer used. The most widely used adsorbent is diethylaminoethyl (DEAE) cellulose, an anion exchanger (Fahey, 1967).

Various workers have employed DEAE-cellulose columns for further purification of chicken Ig' fractions obtained after gel filtration. Higgins (1976) used DEAE sephadex A-50 column and linear gradients of NaCl in 0.015 M tris-HCl buffer, pH 8.0, containing 1 M urea and 1 m M EDTA, while DEAE sephadex G-50 column and linear gradients of phosphate buffer were employed by Goel et al. (1980) and Chhabra and Goel (1980). Nandapalan et al. (1983) used DEAE-52 for further purification of Sephadex G-200 fractionated IgG. Saif and Dorna (1976) employed DEAE cellulose 52 for final purification of turkey IgG and IgM.

Affinity Chromatography

In affinity chromatography, immunoadsorbents, consisting of an insoluble matrix to which either antigen or antibody has been irreversibly bonded without loss of specific combining capacity, are used for the isolation and purification

of antibodies and antigens (Campbell and Velicky, 1967). Of the various matrices used for preparing immunoadsorbents, agarose is the one most commonly used (Garvey et al., 1977).

Immunoadsorbants made from the first peak fraction obtained after gel filtration were used in the purification of chicken IgM and day-old chicken serum of the second peak fraction in the purification of IgG. These were either cross-linked with gluteraldehyde (Higgins, 1976; Goel et al., 1980), or linked to cyanogen bromide-activated sepharose 4B (Goel et al., 1980; Nandapalan et al., 1983).

Toth and Norcross (1981a) prepared duck IgM immunoadsorbant using pooled first three fractions of the ascending part of the first protein peak obtained by gel filtration. Pooled fractions of the second major peak were used for duck IgG immunoadsorbant. A duck IgG immunoadsorbant was also prepared from one-day-old duckling serum. These immunoadsorbants were treated with appropriate volumes of anti-duck IgM and anti-duck IgG sera, to obtain purified duck IgM and IgG. Madge and Ambrosius (1984) isolated duck 7.8 S and 5.7 S antibodies to bovine serum albumin (BSA) from immune sera by an immunoadsorbant technique using BSA cross-linked by gluteraldehyde and glycine-HCl-0.15 M NaCl buffer, pH 2.8, for the elution of specific antibodies from the adsorbant. Finally, several gel filtration steps on sephadex G-200 were carried out for the separation of the 7.8 S from the 5.7 S fractions. Fei

et al. (1986) used sepharose CL-4B conjugated swine IgG to absorb anti-swine IgG antibodies produced in ducks. The duck anti-swine IgG antibody absorbed in the affinity column was desorbed by 0.1 M glycine buffer, pH 2.5. On checking the purity of this antibody by immunoelectrophoresis using rabbit anti-duck whole serum antiserum, only one precipitation line was observed, indicating that the Ig isolated was pure duck IgG.

Electrophoretic studies on serum proteins

Electrophoresis is defined as the movement of charged particles in solution under the influence of an electrical field.

Five precipitin bands were observed in paper electrophoresis and 16 or more bands were seen in starch or acrylamide gel electrophoresis of chicken serum (Ogden et al., 1962; Lunn, 1963; Glick, 1967), pheasant serum (Baker et al., 1966) and in duck serum (Kaminski and Cajos, 1964). Disc electrophoresis of chicken serum revealed 17 serum protein bands and two prealbumins (Glick, 1968). Stratil (1967) also found more than 17 antigens in chicken serum when immunoelectrophoretic studies were done using homologous antisera. "Micro"-2-dimensional immunoelectrophoresis of chicken serum with homologous antisera produced 34 precipitin arcs (Carvella et al., 1977). Singh (1978) identified a total of 38-40 proteins as separate precipitation arcs by crossed immunoelectrophoresis of fowl serum.

The electrophoretic mobility of fowl IgM was found to be in beta 2 or gamma-1 position and it classically produced a 'gull-shaped' arc in immunoelectrophoresis (Asofsky et al., 1962; Patterson et al., 1965; Tureen et al., 1966). Saif and Dohms (1976) reported that turkey serum IgM also migrated in the same position as that of chicken, giving the typical 'gull' shaped arc in immunoelectrophoresis. They also found that turkey IgG in whole serum migrated on immunoelectrophoresis in the gamma through beta position, while purified IgG preparation migrated only in the gamma position.

Unanue and Dixon (1965) reported that analytical ultracentrifuge studies of duck Ig preparations obtained by preparative electrophoresis yielded three peaks with sedimentation coefficient values of 5.89, 7.43 and 16.93. The first two peak proteins migrated in the gamma-2 zone of immunoelectrophoretic test, while the third peak protein migrated in the gamma-1 zone. Grey (1967a) stated that immunoelectrophoresis of starch block isolated gamma-globulin from duck serum showed three antigenicially distinct proteins in the gamma globulin region. One extended directly from the well, similar to mammalian gamma-M globulin, while the other two were located in the region where mammalian gamma-G globulins were usually found on immunoelectrophoresis. Immunoelectrophoretic studies by Grey (1967b) revealed that duck 5.7 S protein formed a precipitin band very close to the antiserum through, while the more antigenically complete and more slowly diffusing

7.8 S protein formed a band behind it. Toth and Norcross (1981a) observed that duck IgM was an electrophoretically heterogeneous protein with components migrating slower than IgM of other species. The cathodal tip of duck IgM lines extended into the gamma-2 migration zone. In immunoelectrophoresis using duck whole serum and its antiserum they also detected that besides the IgM and major IgG arcs, there occurred another arc also in the form of a thin line immediately within the curve of the major IgG line and merging with it towards the cathodal end. This was presumed to be a minor IgG arc. Their studies also revealed that in one-day-old duck serum, a prominent duck IgG arc was developed by both anti-duck serum and anti-duck IgG. But no IgM line was seen. In 14 day old duck serum, a shorter line instead of the typical elongated duck IgG arc appeared in the gamma-2 migration zone close to the trough. This line was recognized as duck IgG by anti-duck IgG. On the well side of this arc, a well-separated weak line not merging with the IgG arc was recognised as duck IgM, by anti-IgM. Similar, but considerably weaker lines were also observed, for the seven-day old duck serum. In a study on the electrophoretic mobility of biliary immunoglobulins of galliforms (chicken and turkey) and anseriforms (duck and goose), Hadge and Ambrosius (1988a) demonstrated that chicken and turkey immunoglobulins were beta-1/alpha-2 globulins while that from duck and goose were beta-2 globulins.

Immunoelectrophoretic studies using sera of bursectomised chicken revealed that upto two weeks of age, the surgically bursectomised and testosterone propionate treated birds exhibited a low Ig level (Brownell et al., 1965). Claflin et al. (1966) also observed decreased levels of IgG and IgM in bursa-deficient chicken serum, by microimmunoelectrophoresis. Hirota and Ito (1975) found that the serum from 14-week-old TP treated chicken developed a longer IgM-precipitin arc than did the normal serum. Electrophoresis of serum from seven-week old Cy treated ducks showed a blotting out of the beta-2 peak and a decrease in gamma globulin level, while the control serum showed a double-peaked pattern at the beta-globulin corresponding site (Hashimoto and Sugimura, 1976).

Quantification of serum proteins

Quantitative methods for the determination of individual serum proteins are important tools in immunological studies. Eventhough the quantitation of chicken serum protein fractions had been dealt with in detail by many workers, there are only very few such reports on the serum protein fractions of ducks. A variety of methods have been employed for the quantitation of avian serum proteins, which include salting out with sodium sulphate, electrophoresis, polyacrylamide gel electrophoresis and radial immunodiffusion. Of these, the single radial immunodiffusion method developed by Mancini et al. (1965) had proved

valuable for quantification of individual immunoglobulins.

Brandt et al. (1951) found by sodium sulphate fractionation method that the serum of four to seven week old chicks contained 3.36 ± 0.25 g/100 ml of protein, of which the gamma globulin value was only 0.40 g/100 ml. The total protein, alpha and garma globulins were found to increase with age, while little difference was noted in albumin or beta globulin fractions. Electrophoresis of normal turkey serum revealed a total protein content of 3.96 to 4.91 g/100 ml. The percentages of the various serum fractions were as follows: albumin 66.5%, alpha-globulin 7.9%, beta globulin 14.4% and gamma globulin 11.2% (Lynch and Stafseth, 1953). Employing paper electrophoresis, Isakovic and Jankovic (1964) demonstrated that the gamma globulin content in surgically bursectomised chicken was significantly lower (0.34 g/100 ml serum) than that in unoperated birds (0.73 g/100 ml serum).

Morgan and Glick (1972) conducted a detailed quantitative study on the serum protein fractions of bursectomised and normal chicken, by polyacrylamide gel electrophoresis. They found that the total serum protein content increased slowly from hatching (2.68 g % at one week) to 12 week of age (4.63 g % at 12 weeks). Neither surgical nor hormonal Bx had any significant effect on the total protein, or albumin levels. Albumin levels rose sharply from hatching until two to three weeks of age. The IgG levels declined significantly

during first two to three weeks and then rose sharply by four to five weeks in control birds, while IgM level in controls was very low or absent at hatching and increased rapidly during the first week. Both surgical and hormonal Bx resulted in a delay in normal IgG production, while the IgM levels in these two groups were higher than control levels. The IgG levels from one to twelve weeks ranged from 350-977 mg % in controls, 373-642 mg % in surgically bursectomised and 417-558 mg % in hormonally bursectomised birds. The IgM levels from one to twelve weeks ranged from 160-414 mg % in controls, 191-567 mg % in surgically bursectomised and 238-574 mg % in hormonally bursectomised birds.

By radial immunodiffusion technique, Lerner et al. (1971) found that at hatching, chicks had about 150 mg % circulating IgG, derived from the yolk. This quantity declined to about 100 mg % by second week, after which the level rose until the 14th week, when a value of 515-550 mg % was attained. In contrast to IgG, no circulating IgM was present at hatching, but after the first week, measurable quantities appeared. IgM level was influenced by specific immunization with sheep cells to a greater extent than were IgG levels. By the 14th week, the mean IgM level of the immunized controls, 400 mg %, was almost twice that of the non-immunized birds, 200 mg %.

Chhabra and Goel (1980) quantitated the immunoglobulins in chicken serum by radial immunodiffusion method and found

mean values of 1.35 mg/ml IgM, 5.09 mg/ml IgG and 0.31 mg/ml IgA.

Sefalco (1942) reported a value of 3.50 g % for total proteins in ducks. By electrophoretic studies, Spector (1956) showed that the plasma of ducks contained more globulins (52.1%) than albumin (47.8%) and that the albumin-globulin ratio was below one. The gamma globulin level was found to be six per cent. Surendranathan (1966) obtained a total protein value of 4.65 ± 0.19 g % in adult male ducks and 5.00 ± 0.14 g % in adult nonlaying female, by micro-kjeldahl method. The mean albumin values were found to be 2.07 ± 0.10 g % in adult male ducks and 2.63 ± 0.75 g % in nonlaying females. The mean globulin values for the above groups were 2.06 ± 0.13 g % and 2.50 ± 0.12 g % respectively. The albumin-globulin ratio in these two groups were 1.10 ± 0.10 and 0.85 ± 0.06 respectively.

Total and differential leucocytic count of ducks

Reports on the normal haematology of ducks are very few, compared to that of chicken.

Magath and Higgins (1934) obtained a count of 23,400 and Sreenivasan and Rao (1965) 37,400 leucocytes per c.mm. of blood in ducks. A differential leucocyte count by Magath and Higgins (1934) revealed an average of 61.7% lymphocytes, 24.3% heterophils, 2.1% eosinophils, 1.5% basophils and 10.6% monocytes. In differential count, Sreenivasan and

Rao (1969) obtained the following mean values of 37.4% lymphocytes, 45.3% heterophils, 6.8% eosinophils, 4.53% basophils and and 7.5% monocytes.

In a study on the normal haematology of ducks, Surendranathan (1966) obtained the total and differential leucocytic counts of ducklings from day old to three month old groups. The total leucocytic count ranged from 28.63 ± 0.63 (in day-old) to 11.03 ± 1.65 (in three month old) thousand per c.mm. of blood. The differential leucocyte counts from day old to three month old ducklings were in the following range: 56.00 ± 0.98% to 64.40 ± 1.37% lymphocytes, 26.10 ± 0.80% to 25.00 ± 1.93% heterophils, 5.20 ± 0.58% to 2.40 ± 0.50% eosinophils, 0.80 ± 0.19% to 0.80 ± 0.16% basophils and 11.00 ± 0.57% to 7.40 ± 0.40% monocytes.

Materials and Methods

MATERIALS AND METHODS

Materials

Protein estimation - biuret reagent

1. Reagent I (alkaline sodium potassium tartarate solution)

$KNaC_4H_4O_6 \cdot 4\ H_2O$	- 12 g
IN NaOH	- 200 ml
KI	- 5 g
Distilled water	- upto 1000 ml

2. Reagent II (5% copper sulphate solution)

$CuSO_4 \cdot 5\ H_2O$	- 5 g
Distilled water	- 100 ml

Precipitation of serum immunoglobulins

1. Saturated ammonium sulphate (SAS) solution

SAS was prepared by adding 760 g of ammonium sulphate (BDH) to one litre of triple distilled water and heating to 50°C for 30 minutes in a water bath, with continuous stirring to dissolve. It was filtered while still hot to remove insoluble impurities and then cooled to room temperature. The pH was adjusted to 7.0 with ammonium hydroxide solution just prior to use.

Working SAS solution

Solution of 66% and 80% strength were prepared (v/v) freshly from the stock SAS.

2. Ammonium hydroxide solution

3. Physiological saline

4. Ten per cent barium chloride solution

5. Borate-buffered saline, pH 8.5

Five parts of borate buffer was added to ninety-five parts of saline.

Borate buffer

Boric acid	= 6.184 g
Borax	= 9.536 g
NaCl	= 4.384 g
Distilled water	= 1000 ml

Added the above reagents to one litre volumetric flask containing 600-800 ml of distilled water and shaken the flask until complete solution of the contents was achieved. Added distilled water to make upto one litre and mixed by additional shaking. Used an aliquot of the solution to check the pH.

6. Crystalline sodium sulphate

Column chromatography

1. Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden)

2. Tris-HCl-NaCl buffer, pH 8.0

Tris (hydroxymethyl) aminomethane 0.1 M (12.11 g/litre)

Sodium chloride 1.0 M (58.45 g/litre) and 0.02% sodium azide in two-third of buffer volume using distilled water. pH was adjusted to 8.0 by adding 1N HCl and the volume was

made upto one litre by distilled water. The buffer solution was filtered through Whatman No.1 filter paper before use.

Immunoelectrophoresis

1. Tris-barbital buffer

Barbitone sodium	- 9.9 g
Tris (hydroxy methyl) aminomethane	- 17.7 g
Sodium azide	- 0.3 g
Distilled water	- 2000 ml

pH adjusted to 8.6 with 1N hydrochloric acid.

2. Agar coated slides

Clean microscope slides (2.5 x 7.5 cm) were dipped in 1% melted agar in distilled water and dried in air by keeping the slides horizontally over glass rods. Dried slides were stored at room temperature until used.

3. Buffered agarose

Agarose, 0.8 g, was boiled in 100 ml Tris-barbital buffer until the agar was dissolved completely and then stored at room temperature until used.

4. Preparation of agarose gel on slides

Agar coated slides were placed on a perfectly horizontal surface and three ml of melted buffered agarose was poured on each slide and allowed to form gel at room temperature.

5. Stain for immunoelectrophorogram

Amido black 10 B	- 1 g
Sodium acetate-acetic acid buffer 0.2 M, pH 3.6	- 1000 ml

6. Decolourising solutions for immunoelectrophoresis

Decolouriser I	
Methanol	- 40 v
Acetic acid	- 10 v
Distilled water	- 10 v
Decolouriser II	
Absolute alcohol	- 35 v
Acetic acid	- 5 v
Distilled water	- 10 v

7. Duck serum samples from experimental and control birds

8. Antiduck serum raised in rabbit

9. Antiduck globulin raised in rabbit

Quantification of IgG and IgM by radial immunodiffusion

1. P.B.S. (pH 7.3)

NaCl	- 8.00 g
K_2HPO_4	- 1.21 g
KH_2PO_4	- 0.34 g
Distilled water	- 1000 ml

2. 1.5% agarose gel in P.B.S.

3. Antiserum to duck IgG and IgM

4. Duck serum samples from experimental and control birds

5. Duck bile

6. Duck egg yolk

Hank's Balanced Salt Solution (HBSS)

A 10 x stock solution was prepared as per the procedure of Cunningham (1966) and stored at 4°C for further use. A 1 x working solution was prepared by diluting the 10 x stock solution with triple distilled water. Penicillin (200 IU per ml) and streptomycin (200 ug per ml) were added to prevent bacterial contamination.

Total count of leucocytes

1. Blood of ducklings anticoagulated with EDTA.

2. WBC diluent: prepared as described by Nath and Harrick (1952).

NaCl	- 0.80 g
Na_2SO_4	- 2.50 g
$Na_2HPO_4 \cdot 12\ H_2O$	- 2.91 g
KH_2PO_4	- 0.25 g
Formalin (37%)	- 7.5 cc
Methyl violet 2B	- 0.100 g

The above chemicals were dissolved in distilled water in the order prescribed and diluted to a total volume of 1000 cc in a volumetric flask. After standing overnight, the solution was filtered through Whatman number 1 filter paper and was ready for immediate use.

3. Haemocytometer

Differential count of leucocytes

1. Blood of ducklings

2. Modified copper peroxidase method: Solution A: 0.5% $CuSO_4$ Solution B: 0.2 g of benzidine was ground in a mortar with a few drops of water. To this was added with constant stirring 200 ml of distilled water, filtered and added four drops of 3% H_2O_2.

S. typhimurium culture

S. typhimurium isolated in this laboratory from guinea pig was used throughout the study.

Sheep erythrocytes (SRBC)

The source of sheep erythrocytes was from the same animal throughout the experiment.

Duck eggs and day-old ducklings

Cross-bred ducklings were obtained from Government Duck Farm, Niranam.

Bile: Bile was aspirated aseptically from control ducklings that were sacrificed at weeks 5, 8, and 10. Pooled bile was kept at -20°C till use.

Endoxan ASTA (cyclophosphamide (P) - obtained from Khandelwal Laboratories Ltd., Bombay.

METHODS

Bursectomy

a) Surgical method

The method described by Chang et al. (1957) was followed. Bursectomy was done on day three after hatching. Pressure was applied to the duckling's back with the left hand and an incision with a razor blade was made at the base of the tail, just above the upper lip of the vent. The bursa was then grasped with curved forceps at the anterior end and eased towards the opening. It was excised as close to its attachment to the cloaca as possible in order to remove all bursa tissue and thus avoid any possible regeneration. Control ducklings were sham bursectomised.

b) Chemical method

Chemical bursectomy was effected by single intramuscular injection of day-old ducklings with cyclophosphamide, at the rate of 2.5 mg/bird.

c) Hormonal method

The method of Glick (1963) was followed for inducing hormonal bursectomy. Five day embryonated duck eggs were dipped in one per cent alcoholic solution of testosterone by the pointed end to a depth of 1½ inches for five seconds. The solution temperature was maintained between 10 and 15°C. Control eggs were dipped in the same manner in ethyl alcohol.

d) Antibursal serum method

Sixteen day embryonated duck embryos were inoculated intravenously with BABS at the rate of 0.1 ml per egg. Control eggs were given 0.1 ml each of physiological saline, intravenously.

Preparation of antigens

Prepared as per the technique described by Chang et al. (1957) with some slight modification.

Salmonella typhimurium

S. typhimurium organism was cultivated on Mueller-Hinton agar medium, in large petri dishes. The culture was harvested with approximately 15 ml of 0.6% formol saline per dish, scrapping with sterile glass rod. It was then filtered through sterile cotton and incubated for 24 h at 37°C. The sterility was tested by inoculating 0.2 ml of this antigen into Mueller-Hinton agar medium and incubating. The stock antigen suspension was kept in the refrigerator.

Working standard of antigen was made by comparing with number 10 McFarland standard (prepared by adding 9 ml of 1 % H_2SO_4 and 1 ml of 1% $BaCl_2$), to get approximately 3000 million organisms per ml. It was further inactivated by keeping at 56°C for 30 mts and was used as the antigen for inoculation. For agglutination test, a concentration of one billion organisms per ml of antigen was used.

Sheep RBC antigen: Sheep red blood cells suspended in Alsever's solution were washed thrice in physiological saline and finally prepared as a 15% saline suspension. This suspension was employed as antigen for inoculations. For serological test, the RBC concentration was reduced to 2% by the addition of physiological saline.

Antibursal serum (ABS)

Antibursal serum was prepared as per the method of Mishra and Jaiswal (1984) with slight modification.

Bursae were collected from four, 4-week old ducklings. They were cut into small pieces and ground in Ten Broeck grinder in MBSS (containing 0.5 per cent lactalbumin hydrolysate and 0.2 per cent yeast extract). It was centrifuged twice in MBSS at 1500 rpm for 10 mts. Then the supernatant was discarded and the cells resuspended in MBSS to get a population density of 10^6 cells per ml. It was then inoculated intravenously into two rabbits, in one ml doses. Three booster injections were given at ten day intervals and the rabbits were bled seven days after the last injection. The serum was separated and centrifuged at 1000 rpm for 10 mts. It was inactivated at 56°C for 30 mts and then treated with 100 units of penicillin and 100 ug of streptomycin sulphate per ml, incubated at 37°C for one hour and kept at -20°C till used.

Experiments to study the immune responses

In order to understand the immune responses against

two different antigens, five sets of experiments were conducted. But for the difference in the category of ducklings used, the protocol and methodology in general, were more or less similar in all the experiments carried out. The two antigens used were aseculture of S. typhimurium having a population density of 3000 million per ml and 15% suspension of SRBC washed in normal saline.

Age matched normal ducklings and bursectomised ones were given single intramuscular inoculation with either one of the above antigens. They were 7 days old, 28 days old and 42 days old ducklings. The dose of S. typhimurium antigen was 0.2 ml for 7 day-old group, 0.5 ml for 28 day-old group and 1 ml for 42 day-old group, while the dose of SRBC antigen was 1 ml for all age groups.

The anticoagulated portion of blood was used for making total and differential leucocyte counts and the blood collected without anticoagulant was used for harvesting serum which was later on used for detecting the antibody levels against the antigens injected. At the end of the fourth week, the ducklings were sacrificed and the blood, bile, spleen and bursa, if present, were collected. The body weights and the weights of bursa and spleen were recorded.

The bursae and spleen were fixed in 10 per cent formol saline for histopathological examination. Tissues were processed by routine paraffin embedding technique. Paraffin

sections cut at 5 micron thickness were stained with Harris haematoxylin and eosin as described by Drury and Wallington (1967).

Experiment I

This group comprised of 8 control ducklings injected with normal saline and two groups of 8 ducklings each injected with either S. typhimurium antigen or SRBC antigen, on days 7, 28 or 42 after hatching. These birds were sacrificed at the end of 4th week post-inoculation and various tissues were collected as described earlier.

Experiment II

Consisted of surgically bursectomised ducklings and sham bursectomised controls. Six bursectomised ducklings inoculated with Salmonella antigen, six bursectomised birds administered SRBC antigen and six controls inoculated with normal saline were there in 7-day age group. In the 28-day age group there were 8 bursectomised ducklings each for salmonella antigen and SRBC antigen and 8 controls inoculated with normal saline. In 42-day age group, four bursectomised ducklings were inoculated with each of salmonella antigen and SRBC antigen and 8 controls inoculated with normal saline.

These birds were sacrificed at the end of the 4th week post-inoculation and the various samples were collected as described earlier.

Experiment III

In this experiment, 8 chemically bursectomised ducklings each for salmonella antigen and SRBC antigen and 8 controls inoculated with normal saline were used for each of 7 days, 28 days and 42 days age groups. These birds were also sacrificed at the end of the 4th week post-inoculation and samples collected as above.

Experiment IV

The birds of 7 days and 42 days age group in this experiment consisted of six hormonally bursectomised ducklings inoculated with salmonella antigen, six Hbx birds inoculated with SRBC antigens and six controls inoculated with normal saline. The birds of 28 days age group comprised of 8 numbers each for salmonella antigen, SRBC antigen and normal saline inoculated controls. At the end of the 4th week post-inoculation these birds were also sacrificed and various samples were collected.

Experiment V

This group comprised of control ducklings given normal saline and ducklings administered with antibursal serum. There were six ducklings under each treatment group and control. As in previous experiments, these birds were also sacrificed at the end of the 4th week post-inoculation and samples collected.

Collection of blood and separation of serum

Pooled sample of blood was collected by sacrificing fifteen ducklings of 8-12 weeks of age. The blood was allowed to stand for half to one hour at room temperature for clot formation, keeping the flasks in a slanting position. Then the clot was carefully separated from the wall of the flasks with sterile rod and allowed to stand at 37°C for one hour for serum separation. Decanted the separated serum into clean, sterile tubes and kept in the refrigerator. The blood clot was also kept in the refrigerator overnight, for further serum separation. The next day the separated serum was clarified by centrifugation at 2000 rpm for 5 minutes and was stored as small aliquotes of one ml in sterile glass vials. All samples were stored with preservative (Merthiolate 1/10000) at -20°C in deep freeze.

Blood from the experimental ducklings of various age groups was collected by cardiac puncture using a 22 gauge needle and serum separated and stored as described above. Collection of blood and separation of serum was also done in the case of ducklings slaughtered at fifth, eighth and tenth weeks.

Estimation of protein concentration

The total protein content in the blood serum was estimated by Biuret method as described by Inchiosa (1964).

Precipitation of globulins in serum

Ammonium sulphate precipitation of globulins

Two final concentrations of ammonium sulphate solution (ASS) of 33% and 40% were used to precipitate the globulins in the pooled serum samples as per the procedure described by Garvey et al. (1977).

With constant stirring using magnetic stirrer, 50 ml of 66 ⅔ or 80% ASS was added dropwise to a 50 ml serum sample. The stirring of serum-ASS mixture was continued for 30 minutes after adding the last drop of ASS and the ensuing precipitate was allowed to stand overnight at 4°C. Then the suspension was centrifuged in a refrigerated centrifuge at 3000 rpm. for 30 min. The precipitate obtained was dissolved in enough saline to reconstitute the original volume of serum and reprecipitated two more times following the above procedure, omitting the overnight keeping of the suspension at 4°C. The precipitate from the third precipitation was dissolved in borate buffered saline to a final volume of 20 ml, i.e., less than half that of the original serum sample. The ammonium sulphate was removed from the precipitate by dialysing against borate buffered saline at 4°C. The saline was changed frequently until there was no ammonium sulphate in the dialysate as evidenced by the absence of turbidity on testing with 10% barium chloride solution.

The concentration of the precipitated proteins was determined by Biuret method (Inchiosa, 1964).

Sodium sulphate precipitation of globulins

Globulins were precipitated from serum at room

temperature as in the above method, by the addition of 18, 14 and 14% sodium sulphate respectively for the first, second and third precipitations. As in the previous case, the concentration of the precipitated proteins was determined by Biuret method of Inchiosa (1964).

Gel filtration chromatography

Gel filtration chromatography was carried out on Sephadex G-200 column using tris-NaCl buffer; pH 8.0 as per the procedure described by Talwar (1993).

Preparation of the column

Sephadex G-200 in 4 g quantity was suspended in enough tris-NaCl buffer at room temperature for three days to ensure proper swelling. The slightly turbid supernatant fluid was removed by decantation to get rid of the fines.

A small piece of glass-wool was placed at the outlet of the glass column having the dimension of 1.5 x 70 cm. It was mounted on a stand in vertical position and filled to about one-third with Tris-HCl buffer pH 8.0. A moderately thick slurry of Sephadex G-200 was poured down the column surface to avoid the trapping of air bubbles. When a 10 cm layer of the gel particles had formed, the capillary outlet was opened. More slurry was added at frequent intervals. When the horizontal zone of packed gel reached a level of 60 cm height, a buffer reservoir was connected. The column

was equilibrated by allowing 2 to 3 column volumes of buffer to pass through the bed.

Preparation of the sample

The test globulin samples were equilibrated by dialysis against the tris-NaCl buffer at 4°C for 24 hours and brought to room temperature before chromatography.

Chromatography

Buffer reservoir was disconnected and the supernatant fluid was allowed to sink almost to the level of gel surface. The equilibrated globulin sample, 1.5 ml, having a total protein concentration of 4.68 mg/ml was loaded very slowly onto the gel without disturbing the gel. As soon as the sample entered the gel, two volumes each of 1.5 ml of the buffer were used to wash in any solution adhering to the column, the first being allowed to sink into the gel before the second portion was used. A few millilitres of buffer was then slowly added. The column was connected to the buffer reservoir and sufficient height of buffer column developed.

The chromatography was conducted manually at room temperature. The flow rate was 14 ml/h and 2 ml fractions were collected.

The protein concentration of each fraction was determined at 280 nm, using a Spectronic-1001. The values

obtained were plotted in graph paper to get the peak curves representing the various proteins in the sample chromatographed. The fractions of the ascending limb of the first major peak and those of the second major peak were subjected to immunoelectrophoresis (described elsewhere) against anti-duck serum raised in rabbit. These fractions were concentrated again using PVP and passed again through the Sephadex column in order to effect further purification of IgM and IgG.

Production of antisera

For each set of antisera two rabbits were used.

a) Rabbit antiduck serum

Two ml of whole duck serum having a protein concentration of 60 mg per ml was homogenized with two ml of Freund's complete adjuvant and two ml each of this emulsion was given intramuscularly to two rabbits. Three booster doses of one ml each, without adjuvants were given at 10 day intervals intramuscularly and the rabbits were bled one week later.

b) Rabbit antiduck globulin (RADG)

Rabbit antiduck globulin was raised by the same procedure as for RADS, using $(NH_4)_2SO_4$ precipitated serum globulins dissolved in borate-buffered saline and having an approximate protein concentration of 10 mg/ml.

c) Rabbit anti-IgM (RAM)

Purified IgM fraction 3 ml (having a protein concentration of 0.35 mg per ml) was homogenised with 3 ml of Freund's complete adjuvant and 3 ml each of the emulsion was inoculated intramuscularly to two rabbits. Two booster doses of 1.5 ml each of IgM alone, without adjuvant, were given at weekly intervals and the rabbits were bled one week later.

d) Rabbit anti-IgG (RAG)

Rabbit anti-IgG serum was produced by the same method as for RAM, using purified IgG having a protein concentration of 0.22 mg/ml.

Immunoelectrophoresis

Melted 0.8% agarose in tris-barbiturate buffer and poured 3 ml of the hot agarose onto each slide kept on a levelled surface. Allowed the agar to harden for 30 mts at 4°C. Wells and troughs were cut on each slide and the agar was sucked out from the wells only, using a vacuum pump. The wells were filled with antigens and a drop of bromphenol blue dye was added to the side of the well as indicator. The slides were then placed in the electrophoresis chamber in such a way that the antigen wells were nearer to the cathode than to anode. Contact between the slides and the buffer was effected by filter paper wicks.

one on each end of the slide, so that each covered about 1/2 cm of the agarose on the slide. Current at the rate of 3 mA per slide was given and the electrophoresis was continued till the indicator dye reached 1 cm away from the other end of the slide. The slides were taken, the agarose in the troughs were removed carefully and the troughs were filled with the respective antisera (Raising of antisera described elsewhere). Allowed the antisera to diffuse 20-24 h at room temperature, keeping the slides in the electrophoretic chamber itself. The slides were then washed by soaking in two changes of normal saline for 24 h and then in distilled water for further 24 h to remove unreacted excess proteins. The slides were dried slowly, stained with amidoblack stain for 15 mts, and decolourised in solutions I and II for 20 mts each. Dried the slides at 37°C for 1 h and mounted in DPX.

Serological tests

The methods described by Chang et al. (1957) with slight modification were followed.

a) Bacterial agglutination to detect antibody against S. typhimurium

To 0.40 ml aliquots of serial dilutions of serum (1/8 to 1/4096) were added to 0.40 ml of the standard bacterial antigen (1 billion organisms per ml). After incubation at 37°C for 24 h, the agglutinin titre of the

test serum was ascertained as the last serum dilution tube containing visible aggregation. The titre was expressed as the reciprocal of this dilution.

b) Sheep red blood cell agglutination to detect hemolysin

Serial saline dilutions of each test serum (1/8 to 1/4096) were prepared in Perspex plates in 0.4 ml aliquotes. An equal quantity (0.4 ml) of sheep erythrocyte suspension (2.0%) was introduced into each well in the dilution series. A saline-sheep red blood cell control was included with each titration. The reactants were mixed by shaking and incubated in a 37°C incubator for 3 h. The titration end point was determined as the greatest serum dilution in which significant agglutination of erythrocytes was still discernible. The titre was expressed as the reciprocal of this dilution.

Quantitation of IgG and IgM

Single radial immunodiffusion (SRID) techniques, developed by Mancini et al. (1965) with slight modification was used.

Agarose gel (1.5%) in PBS was melted and kept at 56°C in a water bath. Anti-serum against IgG and IgM, warmed to 56°C, was added to the agarose gel to obtain a final concentration of 5% of antiserum in the gel. These mixtures in 3 ml quantities were overlayered in agar-coated slides.

After solidification, wells of 3 mm diameter were punched out at a distance of 12 mm between the wells. The wells were then charged with 10 ul of varying dilutions of IgG and IgM of known protein concentration and incubated at 4°C for 24 h in humid chamber.

Antigen-antibody precipitation rings formed around the wells were observed and the diameters measured. The slides were washed and stained as in the case of immunoelectrophoretograms. Duplicate determinations of the precipitation ring diameters were made and average values were taken to construct the standard curve, plotting the ring diameters against corresponding protein concentrations of antigen. This curve was used for calculation of Ig concentration in the test sample.

The serum samples from the control and treated ducklings of various experimental groups (described infra) and bile and egg yolk from normal healthy birds were used as test samples. The serum and bile samples were diluted 5 times to ensure that the ring diameters were within the range of diameters produced by standard reference antigens. Egg yolk from six duck eggs diluted 1/4 with PBS was used to fill the antigen well for quantification of IgG and IgM.

Total WBC count (T.C.)

The method of Nath and Harrick (1952), with slight modification, was used.

Differential count

Modified copper peroxidase method of Sato and Sekiya (1965) was followed.

Statistical analysis

Statistical analysis of the data was done by the method of Snedecor and Cochran (1967).

Results

RESULTS

Experiments to study the immune responses

The immune responses to SRBC/*S. typhimurium* were studied in non-bursectomised (control) and bursectomised ducklings Bursectomy was performed by surgical, chemical, hormonal or antibursal serum methods. The antigen (SRBC/*S. typhimurium*) was inoculated at day 7, 28 or 42 of age and the responses in body weight and weights of bursa and spleen were determined, after sacrificing the birds four weeks after giving the antigen, viz., at fifth, eighth and tenth weeks respectively. The body weights, as well as the weights of bursa and spleen in the control and treatment groups were analysed by Analysis of Variance.

Comparison between the body weights, weights of bursa and spleen in non-bursectomised control and antigen inoculated ducklings

At the fifth week of age

In the control group the maximum body weight obtained was 635 g and the minimum was 485 g, while in SRBC treated group the maximum was 760 g and the minimum was 475 g. In *S. typhimurium* inoculated group the maximum body weight was 650 g and the minimum was 515 g. Eventhough the weights obtained in the treated groups were higher than that of the control, statistical analysis indicated that the differences were not significant.

The weight of bursa in control group ranged from 0.940 to 1.332 g. In SRBC treated group the weight ranged from 0.548 to 1.333 g, while in S. typhimurium given group it was 0.689 to 1.211 g. Statistical analysis did not reveal any differences in the weights of bursa between control and treated groups.

At fifth week, the weight of spleen in control ducklings ranged from 0.397 —1.129 g, while the range in the SRBC treated group was 0.270 —1.125 g and that in the S.typhimurium given group was 0.393 —0.994 g. In this case also, the weights of spleen in the three groups were not found to be statistically significant.

At the eighth week of age

At eighth week of age, the body weight in the control ducklings ranged from 740-1000 g while that in the SRBC group was 540 —1070 g and in S. typhimurium given group it was 690 —850 g. Statistically significant difference ($P < 0.05$) was observed between the mean body weights of S. typhimurium treated (751.875 g) and the control (865 g) and SRBC given (882.5 g) groups (Table 1).

The weights of bursa at eighth week of age ranged in control group from 0.706 —1.310 g. In SRBC given group it was 0.669 —1.465 g, while in S. typhimurium group it was 0.800 —1.239 g. Statistically there was no significant difference between these groups.

Table 1. ANOVA table to find out differences in body weight between 8 week-old control and SRBC/S. *typhimurium* inoculated ducklings

Source	DF	SS	MS	F	Inference
Treatments	2	80443	40221.5	3.538578	*
Error	12	238698	11366.57		

* Significant ($P < 0.05$)

In control ducklings, weight of spleen at eight week ranged from 0.501 to 0.790 g. In SRBC group it was 0.424 -1.277 g and in S. typhimurium group it was 0.398 - 0.664 g. On statistical analysis, the values were found to be non-significant.

At the 10th week of age

In the 10th week of age, the body weight of control duckling had a range from 990 -1320 g while that for SRBC group was 850 -1240 g and for the S. typhimurium group, 640 -1260 g.

Weight of bursa in control ranged from 0.066 -1.455 g. In the treated groups, SRBC had a range from 0.892 -1.610 g, while the range for S. typhimurium group was from 0.615 - 1.610 g.

The ranges of splenic weights in the three groups were as follows. Control 0.457 -0.860 g, SRBC 0.431 to 0.691 g, S. typhimurium - 0.399-1.610 g.

Statistically there were no significant differences between the body weights and the weights of bursa and spleen of the three groups.

Comparison of the body weights and weights of bursa and spleen between non-bursectomised and surgically bursectomised ducklings inoculated with SRBC/S. typhimurium

In this case, there were four groups - non-bursectomised uninoculated control, surgically bursectomised uninoculated

control,(SBxC), surgically bursectomised and SRBC treated group (SBxSR) and surgically bursectomised and S. typhimurium given group (SBxSt).

At the fifth week

The body weights in control group ranged from 475 g-805 g, while it was 520-709 g in SBxC, 345-935 g in SBxSR and 310-775 g in SBxSt.

Weight of bursa in control ranged from 1.032-1.699 g, while bursa was completely absent in the other groups.

In the case of splenic weight, the control had a range of 0.440-0.777g, while in others the ranges were 0.420-0.747 g (SBxC), 0.320-1.276 g (SBxSR) and 0.191-0.774 g (SBxSt).

Analysis of Variance showed that the differences in body weight and weight of spleen were not significant.

At the eighth week

In control ducklings, the body weights at eighth week of age ranged from 600-920 g. In SBxC the range was 590-940 g, in SBxSR it was 350-810 g and in SBxSt, 430-760 g.

The weight of bursa in control ranged from 0.469-1.380 g, while bursa was not detected in other groups.

Control ducklings had a splenic weight in the range of 0.017-0.674 g, while in others the ranges were 0.235-0.474 g, (SBxC), 0.121-0.367 g (in SBxSR) and 0.128 to 0.348 g (in SBxSt).

Statistical analysis revealed no significant difference in body weight between control and treated groups. But significant differences were noticed ($P < 0.01$) in mean splenic weight between control (0.498 g) and the three treatments of SBxC (0.316 g), SBxSR (0.219 g) and SBxSt (0.279 g) (Table 2).

At the tenth week

At tenth week of age, body weight of control ducklings had a range of 600-1270 g, while the ranges in treated groups were as follows: SBxC (480-895 g), SBxSR (560-1160 g) and SBxSt (600-1070 g).

The weight of bursa in control ranged from 0.265-1.414 g, while bursa was absent in the treated groups.

With regard to the splenic weight, the ranges were 0.022-1.092 g (control), 0.217-2.112 g (SBxC), 0.278-1.307 g (SBxSR) and 0.256-0.842 g (SBxSt).

The values in the case of body weight and splenic weight between the groups were found to be not significant, by Analysis of Variance.

Comparison of the body weights and weights of bursa and spleen between non-bursectomised and cyclophosphamide treated ducklings inoculated with SRBC/S. typhimurium

As in the previous cases, this experiment also contained four groups - non-bursectomised and uninoculated control, cyclophosphamide treated and uninoculated ducklings (CyC) cyclophosphamide treated ducklings inoculated with SRBC

Table 2. ANOVA table to find out differences in weight of spleen between 8-week-old control and surgically bursectomised ducklings inoculated with SRBC/*S. typhimurium*

Source	DF	SS	MS	F	Inference
Treatments	3	1392417	464139.1	13.83469	**
Error	20	670978.8	33548.94		

** Significant ($P < 0.01$)

(CySR) and cyclophosphamide treated ducklings inoculated with S. typhimurium (CySt).

At the fifth week

In control ducklings, body weight ranged from 485-635 g. In the Cy treated ducklings the ranges were 460-620 g (CyC), 460-655 g (CySR) and 485-600 g (CySt).

The weight of bursa was in the following ranges: 0.540-1.013 g (control), 0.285-0.500 g (CyC), 0.255-1.063 g (CySR) and 0.242-0.636 g (CySt).

In control ducklings, splenic weights varied from 0.397-1.129 g, while it ranged from 0.380-0.850 g (in CyC); 0.262-0.892 g (in CySR); and 0.751-1.287 g (in CySt).

Analysis of Variance showed significant differences ($P < 0.01$) between the mean bursal weights of control (0.955 g) and other groups of CyC (0.393 g), CySR (0.457 g) and CySt (0.396 g). The differences in body weights and splenic weights of the four groups were not statistically significant (Table 3).

At the eighth week

Body weight of control ducklings had a range of 1000-1390 g, while in others the ranges were 455-770 g (CyC), 960-1165 g (CySR)and 920-1225 g (CySt).

The weight of bursa showed the following ranges in the four groups - 0.812-1.461 g (control), 0.210-0.448 g (CyC) 0.218-1.025 g (CySR) and 0.361-1.013 g (CySt).

Table 3. ANOVA table to find out differences in weight of bursa between 5-week-old control and Cy-treated ducklings inoculated with STDC/3. typhimurium

Source	DF	SS	MS	F	Inference
Treatments	3	1.658007	0.5526689	11.96287	**
Error	24	1.108769	4.6198695-02		

** Significant ($P < 0.01$)

With regard to the weight of spleen, the ranges were as follows: 0.444-1.008 g (control), 0.141-0.589 g (CyC), 0.507-1.071 g (CySR) and 0.224-0.852 g (CySt).

Statistical analysis by Analysis of Variance revealed significant differences ($P<0.01$) in mean body weights, between the control (1240 g) and the groups-CyC (598.125 g), CySR (1055 g) and CySt (1109.167 g). The body weights between CyC and the other two treated groups of CySR and CySt were also significantly different (Table 4).

Differences in the weight of bursa between the control (1.038 g) and the other groups of CyC (0.314 g), CySR (0.492 g) and CySt (0.577 g) were also statistically significant ($P<0.01$). (Table 5).

With regard to the weight of spleen, significant difference ($P<0.01$) was observed between the control (0.806 g) and CyC (0.270 g) and also between CyC and the other two treatments of CySR (0.771 g) and CySt (0.641 g) (Table 6).

<u>At the tenth week</u>

At tenth week of age, body weight ranged from 600-1270 g (control), 555-1290 g (CyC), 620-900 g (CySR) and 610-645 g (CySt).

Weight of bursa had a range of 0.265-1.308 g (control), 0.272-1.236 g (CyC), 0.135-0.454 g (CySR) and 0.140-0.230 g (CySt) (Plate 1).

Table 4. ANOVA table to find out differences in body weight between 8 week-old control and Cy-treated ducklings inoculated with SRBC/*S. typhimurium*

Source	DF	SS	MS	F	Inference
Treatments	3	1843266	614422	49.27739	**
Error	25	311716	12468.64		

** Significant (P < 0.01)

Table 5. ANOVA table to find out differences in weight of bursa between 8 week-old control and Cy-treated ducklings inoculated with SRBC/*S. typhimurium*

Source	DF	SS	MS	F	Inference
Treatments	3	2.265476	0.7551588	15.00771	**
Error	25	1.257952	5.031807E-02		

** Significant (P < 0.01)

Table 6. ANOVA table to find out differences in weight of spleen between 8 week-old control and Cy-treated ducklings inoculated with SRBC/*S. typhimurium*

Source	DF	SS	MS	F	Inference
Treatments	3	1.420243	0.4734144	12.03744	**
Error	25	0.9832125	0.0393285		

** Significant (P < 0.01)

Plate 1. Ten week-old burns of control and treated ducklings


TEN WEEK OLD BURSA
CONTROL
CYCLOPHOSPHAMIDE TREATED
TESTOSTERONE TREATED
ANT BURSAL SERUM TREATED

Splenic weight ranged from 0.022-1.092 g (control), 0.202-1.316 g (CyC), 0.112-0.424 g (CySR) and 0.115-0.249 g (CySt).

Analysis of Variance indicated significant difference ($P < 0.05$) only in weight of bursa. The differences in body weight and weight of spleen between the groups were not significant. Bursal weight showed significant differences between the control (0.709 g) and two treatment groups of CySR (0.249 g) and CySt (0.195 g). Significant differences in bursal weight were also observed between CyC (0.783 g) and the two treatments of CySR and CySt (Table 7).

Comparison of the body weights and weights of bursa and spleen between non-bursectomised and testosterone treated ducklings inoculated with SRBC/S. typhimurium

In this experiment also, four groups were there - non-bursectomised uninoculated control, testosterone treated uninoculated control (TC), testosterone treated SRBC inoculated ducklings (TSR) and testosterone treated S. typhimurium inoculated ducklings (TSt).

At the fifth week

The body weight of control ducklings at fifth week ranged from 390-450 g. In the treated groups the ranges were as follows: 250-410 g (TC), 230-530 g (TSR) and 210-470 g (TSt).

Weight of bursa was in the range of 0.287-0.570 g

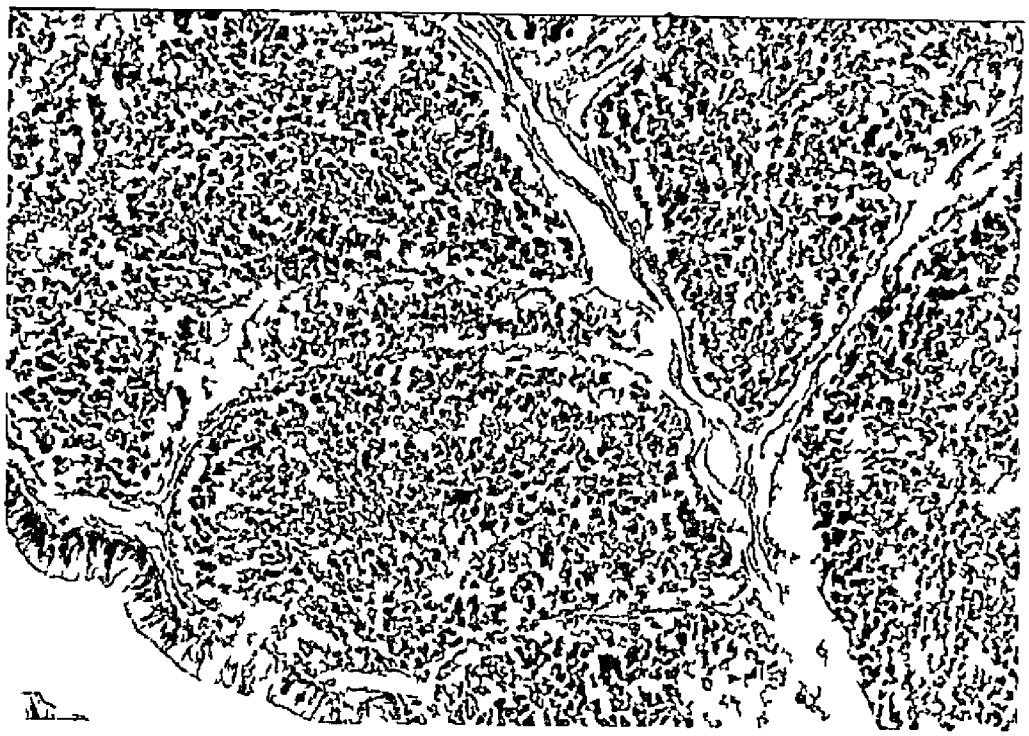
Table 7. ANOVA table to find out differences in weight of bursa between 10 week-old control and Cy-treated ducklings inoculated with SRBC/*S. typhimurium*

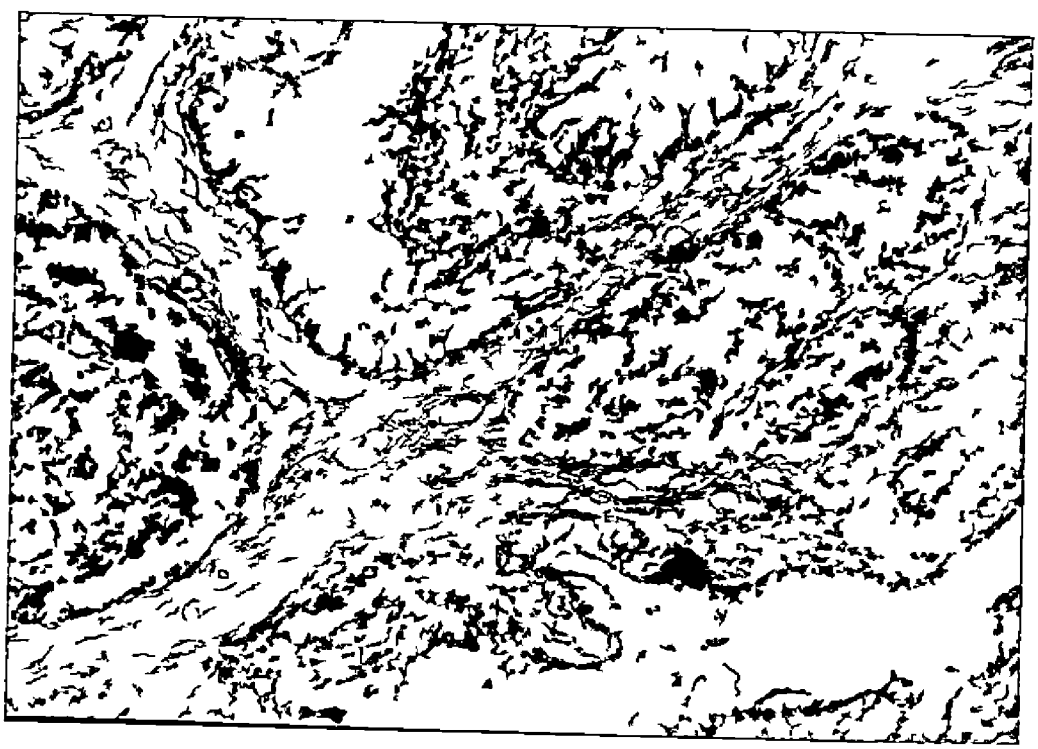
Source	DF	SS	MS	F	Inference
Treatments	3	1.31987	0.4399567	4.560891	*
Error	17	1.639869	9.646237E-02		

* Significant ($P < 0.05$)

Plate 2. Bursa of non-bursectomised SRBC inoculated duckling: Well defined lymphoid nodules containing loosely arranged lymphocytes encapsulated by thick bands of fibrous tissue. Surface epithelium intact.
H & E. X 250

Plate 3. Bursa of cyclophosphamide treated SRBC inoculated duckling: Scattered lymphoid follicles with loosely distributed lymphoid cells. Epithelial cells predominant and stroma oedematous.
H & E X 250





(control), 0.225-0.435 g (TC), 0.232-0.442 g (TSR) and 0.221-0.730 g (TSt).

Splenic weights ranged from 0.209-0.270 g (in control), 0.145-0.350 g (in TC), 0.080-0.446 g (in TSR) and 0.138-0.444 g (in TSt).

Analysis of Variance revealed that the differences in body weight, weight of bursa and weight of spleen between the control and treated groups were not significant.

At the eighth week

In control ducklings, the body weight at eighth week ranged from 690-925 g. The treated ducklings showed the ranges of 420-929 g (in TC), 255-880 g (in TSR) and 530-1090 g (in TSt).

The range in the weight of bursa was from 0.533-1.343 g in control, 0.227-0.955 g in TC, 0.110-0.647 g in TSR and 0.302-0.970 g in TSt.

Weight of spleen was in the range of 0.368-0.725 g (in control), 0.270-0.538 g (in TC), 0.162-1.077 g (in TSR) and 0.315-0.747 g (in TSt).

Statistical analysis showed that there were no significant differences in the control and treated groups, between the body weights, weights of bursa and spleen.

At the tenth week

At tenth week of age, control ducklings recorded a minimum

body weight of 620 g and a maximum of 920 g. In TC, the minimum body weight was 880 g and the maximum was 980 g. The lowest body weight in TSR was 910 g and the highest was 1065 g. In TSt group, body weight ranged from 785-1100 g.

The weights of bursa ranged from 0.578-1.276 g (in control), 0.484-1.020 g (in TC), 0.467-0.989 g (in TSR) and 0.329-1.206 g (in TSt).

Splenic weights in control and treatments groups were in the following ranges: 0.674-1.676 g (control), 0.574-1.180 g (TC), 0.499-1.417 g (TSR) and 0.592-1.004 g (TSt).

Analysis of Variance revealed significant differences in mean body weight ($P < 0.05$) between the control (777.8 g) and two treatment groups, viz., TC (918 g) and TSR (961 g) (Table 8). Differences in the weights of bursa and spleen were not significant.

Comparison of the body weight, weight of bursa and spleen between non-bursectomised and antibursal serum treated ducklings inoculated with SRBC/S. typhimurium

This experiment also consisted of four groups of ducklings as in the previous cases. The first group comprised of non-bursectomised, uninoculated control, followed by antibursal serum treated uninoculated group (ABC), antibursal serum administered ducklings inoculated with SRBC (ABSR) and antibursal serum given ducklings inoculated with S. typhimurium (ABSt). Comparison of the body weight, weights of bursa and

Table 8. ANOVA table to find out differences in body weight between 10 week-old control and testosterone treated ducklings inoculated with SRBC/ S. typhimurium

Source	DF	SS	MS	F	Inference
Treatments	3	102337	34112.33	3.652076	*
Error	15	140108	9340.533		

* Significant ($P < 0.05$)

spleen, was done at three age groups of fifth week, eighth week and tenth week.

At the fifth week

The body weight of the control ducklings ranged from 380-450 g, while that of the treated groups ranged from 280-520 g (ABC), 280-375 g (ABSR) and 245-435 g (ABSt).

Weights of bursa showed a range of 0.247-0.555 g (control), 0.331-0.779 g (ABC), 0.343-0.671 g (ABSR) and 0.259-0.645 g (ABSt).

The weight of spleen in control ducklings had a range of 0.209-0.270 g, while the ranges in the other groups were 0.250-0.370 g (ABC), 0.168-0.350 g (ABSR) and 0.155-0.288 g (ABSt).

The differences in body weight and in weight of bursa and spleen between control and treated groups were not significant as per Analysis of Variance.

At the eighth week

At the eighth week, control ducklings recorded a body weight range of 820-865 g, while the ranges in treated groups were, 595-770 g (ABC), 423-780 g (ABSR) and 445-860 g (ABSt).

The ranges in the weight of bursa were as follows: 0.462-1.194 g (control), 0.922-1.697 g (ABC), 0.320-0.755 g (ABSR) and 0.340-1.027 g (ABSt).

Weights of spleen ranged from 0.521-0.855 g (control)

0.601-1.364 g (ABC), 0.423-1.740 g (ABSR) and 0.250-0.725 g (ABSt).

Statistical analysis revealed significant differences in mean body weight ($P < 0.05$) between control (843.33 g) and the treated ducklings, viz., ABC (704.167 g), ABSR (624.167 g) and ABSt (696.667 g) (Table 9).

Significant differences ($P < 0.05$) were also observed in mean bursal weight between ABSR (0.319 g) and the control (0.870 g) and also between ABSR and ABC (1.050 g) (Table 10). Differences in weight of spleen between the control and treated groups were found to be non-significant.

At the tenth week

The body weights in control and antibursal serum treated ducklings were in the following ranges: 560-915 g (control), 495-900 g (ABC), 910-1100 g (ABSR) and 830-935 g (ABSt).

Weights of bursa ranged from 0.305-1.051 g (control), 0.430-1.071 g (ABC), 0.640-1.140 g (ABSR) and 0.668-1.144 g (ABSt).

Splenic weights had a range of 0.279-0.948 g (control), 0.338-0.957 g (ABC), 0.425-0.875 g (ABSR) and 0.428-0.757 g in ABSt.

Analysis of Variance revealed significant differences ($P < 0.05$) between the mean body weights of ABSR group (976 g) and that of control (787.5 g) and ABC (739 g) (Table 11). Differences in weights of bursa and spleen between the control and treatment groups were not statistically significant.

Table 9. ANOVA table to find out the differences in body weight between 8 week-old control and antibursal serum treated ducklings inoculated with SRBC/ S. typhimurium

Source	DF	SS	MS	F	Inference
Treatments	3	150938	50312.67	3.962283	*
Error	20	253958	12697.9		

* Significant (P < 0.05)

Table 10. ANOVA table to find out the difference in weight of bursa between 8 week-old control and antibursal serum treated ducklings inoculated with SRBC/ S. typhimurium

Source	DF	SS	MS	F	Inference
Treatments	3	0.8921538	0.2973846	4.628936	*
Error	20	1.284922	6.424608E-02		

* Significant (P < 0.05)

Table 11. ANOVA table to find out the differences in body weight between 10 week-old control and antibursal serum treated ducklings inoculated with SRBC/ S. typhimurium

Source	DF	SS	MS	F	Inference
Treatments	3	167483	55827.67	3.849654	*
Error	17	246598	14505.77		

* Significant (P < 0.05)

Histopathology

Bursa of Fabricius

The bursa of Fabricius was completely absent in all the birds which were surgically bursectomised. The following histopathological changes were noticed in the bursa of non-bursectomised and bursectomised groups.

Sheep RBC treated group

Non-bursectomised birds given only sheep RBC as antigen showed, at five weeks, well defined lymphoid nodules containing loosely arranged lymphocytes encapsulated by thick bands of fibrous tissue. Surface epithelium was intact (Plate 2). There was no germinal centre activity in lymphoid follicles even though heterophilic infiltration was seen in some of the follicles. By eight weeks, lymphoid follicles showed germinal centre activity and macrophage response. Epithelial cells were prominent and there was a tendency for crypt formation. By ten weeks, very active lymphoid follicles with active germinal centres were present, showing macrophage response.

S. typhimurium treated group

Bursa of birds given S. typhimurium alone showed at five weeks, many active lymphoid follicles encapsulated by thin bands of fibrous tissue and containing loosely arranged lymphoid cells. Generally there was no germinal centre activity even though macrophage response was present in germinal

centres of certain follicles. Epithelial lining was intact. At eight weeks, active lymphoid follicles were present, and some of the germinal centres showed macrophage and histiocyte response. Proliferation of macrophages occurred in perilymphoid locations. By ten weeks, many active lymphoid follicles with widened, active germinal centres were present. Lymphoid cells showed diffuse proliferation and there was focal macrophage reaction in some areas. In the perilymphoid locations, reticular cell proliferation was seen. Epithelial lining was intact and there was stromal oedema.

Cyclophosphamide treated group

Extensive dwarfing and thinning of the bursal folds, with severe degree of crypt formation, was seen in five week old cyclophosphamide treated uninoculated birds. The prominent, long villous appearance of the epithelial surface was very characteristic. The number of follicles was few and those which were present were compact, with loosely arranged lymphoid cells. There was relatively more amount of reticular cells. Sub-surface epithelial cells were active. Heterophilic and macrophagic infiltration occurred in sub-epithelial tissue. In certain areas, there was necrosis in the sub-epithelial region. Epithelial layer was thrown into long, thin papillary folds in eight week old bursa. Stroma was abundant and only focal loose lymphoid collections and scattered plasma cells were seen. Lymphoid follicles were

few in number and contained very loosely arranged lymphoid cells. In some follicles, there was degeneration and necrosis of lymphoid cells. Ten week old bursa showed atrophy of lymphoid follicles. The lymphocytes in the follicles were loosely arranged. Prominent epithelial folds were present and reticular hyperplasia was also seen.

Bursa of cyclophosphamide and sheep RBC treated birds showed at five weeks only very few, scattered lymphoid follicles with loosely disseminated lymphoid cells. Epithelial cells were predominant and the stroma was oedematous. Surface epithelium showed invagination and crypt formation. In certain areas, there was desquamation of epithelial lining cells (Plate 3). At eight weeks, lymphoid follicles were hypertrophic and contained loosely arranged lymphoid cells. Proliferating lymphoid cells were seen towards the periphery of the lymphoid follicles. Epithelial cells were prominent. Ten week old bursa showed active lymphoid follicles with active germinal centres and proliferation of lymphocytes. There was interstitial oedema and a tendency for crypt formation.

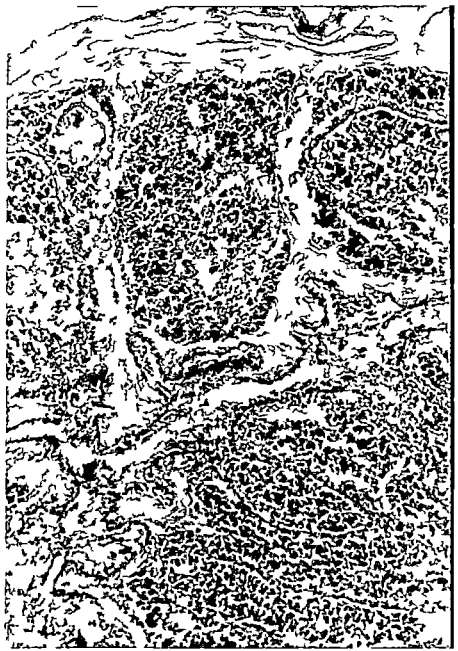
Many lymphoid follicles consisting of loosely arranged lymphoid cells were present in the bursa of five week old cyclophosphamide and S. typhimurium treated group of birds. Surface epithelial cells showed desquamation, invagination and formation of crypts. Stroma was oedematous. In some follicles, germinal centres showed macrophage response. At eight weeks, many active follicles were present and lymphoid

cells showed diffuse proliferation. Germinal centres were active and widened (Plate 4). Numerous lymphoid follicles with loosely arranged lymphocytes were seen with bursa at ten weeks. Certain follicles had active germinal centres. Epithelial cells were prominent and there was a tendency for crypt formation.

Testosterone treated group

In testosterone treated uninoculated ducklings, at five weeks of age, there was stromal oedema with focal areas of degeneration and necrosis. The epithelial cells showed degeneration which was not seen at both eight week and ten week old controls (Plate 5). At eight weeks, the epithelial lining was intact and at ten weeks, epithelial cells were very prominent. In all the three age groups of five, eight and ten weeks, lymphoid follicles contained loosely arranged lymphoid cells. At five weeks, the lymphocytes in some follicles showed karyorrhexis and karyolysis. The stroma was abundant at ten weeks, with slight stromal oedema.

Testosterone and sheep RBC treated birds at five weeks revealed bursa having many follicles with slightly activated germinal centres. The lymphoid tissue contained hypertrophic and hyperchromatic lymphoid cells, some of which were in stages of mitosis. There was slight congestion of blood vessels and in focal areas in the submucosal layer, areas of heterophil and macrophage reaction were seen (Plate 6).



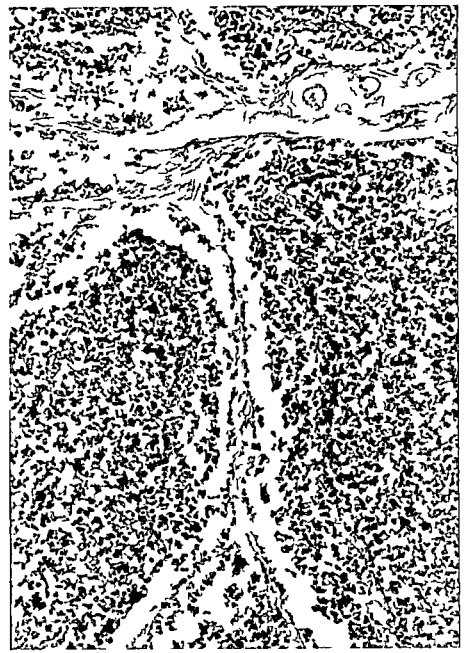
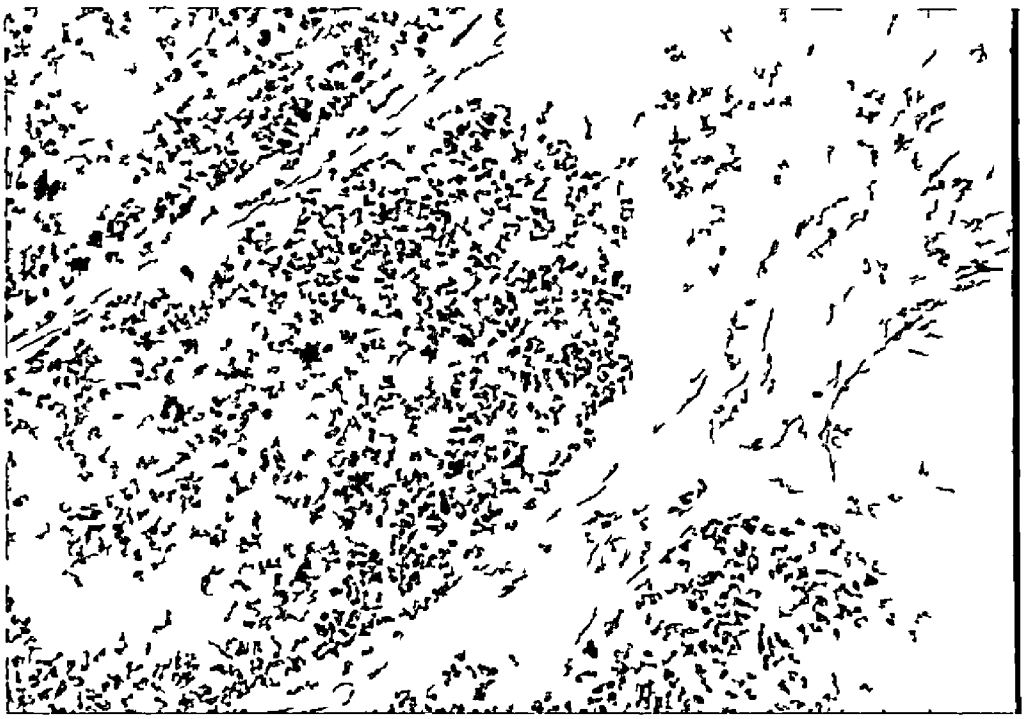


Plate 6. Bursa of testosterone treated SRBC inoculated duckling: Lymphoid tissue show hypertrophic and hyperchromatic lymphoid cells. Areas of heterophil and macrophage reaction.
H & E. X 160

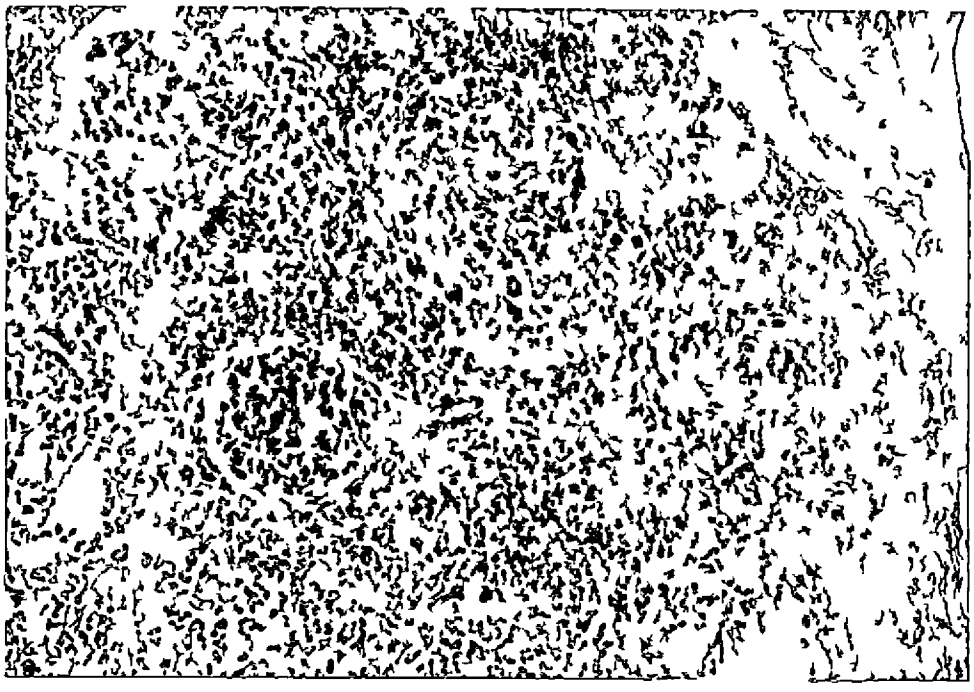
Plate 7. Bursa of testosterone treated S. typhimurium inoculated duckling: Numerous well developed follicles with active germinal centres. Severe macrophage reaction seen towards periphery of lymphoid follicles. Mild stromal oedema.
H & E. X 250

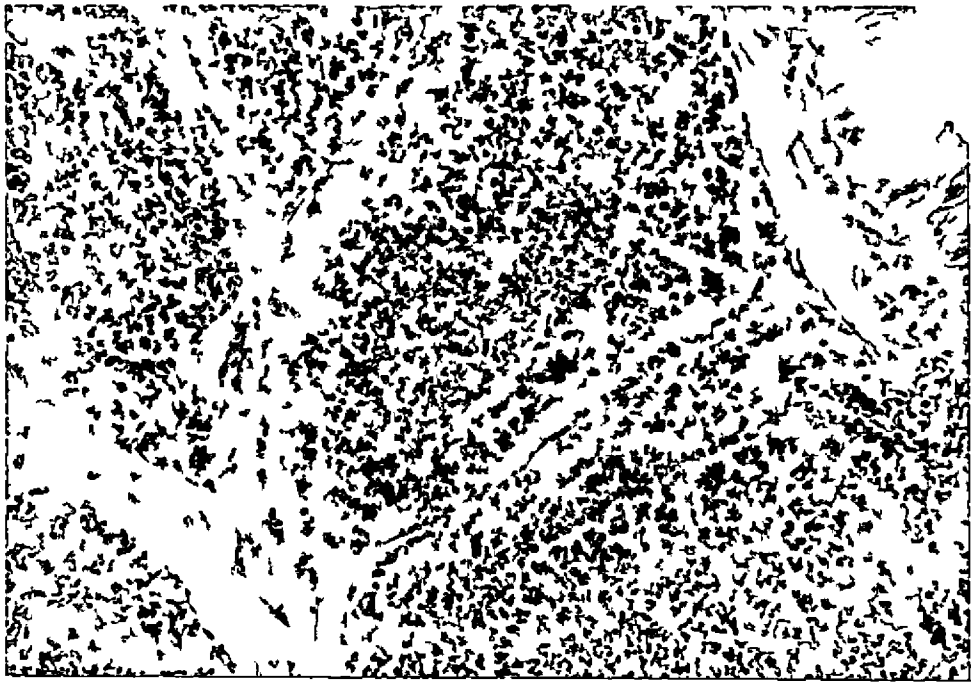


loosely arranged lymphoid cells, but the germinal centres were active.

Antibursal serum treated and sheep RBC given group showed well encapsulated lymphoid nodules with very loosely arranged lymphoid cells, at five weeks of age. There was a tendency for necrosis in some of the lymphoid nodules, especially in the follicles in subepithelial location. Diffuse proliferation of lymphocytes occurred without germinal centre formation, and in focal areas there was proliferation of macrophages. By eight weeks, lymphoid follicles showed diffuse lymphoid collections, still without germinal centre activity. Stroma showed slight oedema and in certain areas, hyalinisation and necrosis. Many lymphoid follicles had active germinal centres and diffusely proliferating lymphocytes at ten weeks. There was also a tendency for crypt formation. Peripheral lymphoid cells were severely hyperchromatic and active. There were numerous follicles with well developed germinal centres. Slight stromal oedema was also present.

The burse of the five week old group which was given antibursal serum and administered S. typhimurium antigen revealed loosely arranged lymphoid follicles some of which showed diffuse heterophil infiltration (Plate 8). The lymphoid follicles were hypertrophic and showed active germinal centres. The lumen of the burse contained degenerated desquamated epithelial cells. Surface epithelial cells also





lymphoid follicles with active germinal centres were also present at ten weeks (Plate 9).

Surgically bursectomised group

Bursectomised uninoculated birds at five weeks showed focal areas of reticular cell hyperplasia in the spleen. Lymphoid follicles were inactive even at ten weeks, and contained diffusely arranged, loose collection of lymphocytes.

Spleen of bursectomised and sheep RBC given birds showed only slight reticular cell hyperplasia and focal areas of lymphoid hyperplasia at five weeks of age. At eight weeks also there was slight reticular cell hyperplasia. Ten week old spleen showed congestion and diffuse lymphoid hyperplasia. No follicles were seen.

S. typhimurium treated bursectomised birds showed focal and diffuse hyperplasia of lymphocytes at five weeks. There was moderate to severe reticular cell hyperplasia. Diffuse proliferation of lymphocytes and reticular cells in the periarterial sheath was observed at eight weeks. There were also many macrophages in periarterial locations.

Cyclophosphamide treated group

The spleen of five-week old cyclophosphamide treated uninoculated birds showed depletion of lymphoid cells and loosely arranged lymphoid aggregates without follicle formation.

At five weeks of age, cyclophosphamide and sheep RBC treated group showed diffuse lymphoid hyperplasia. There were few lymphoid follicles with active germinal centres. Focal areas of reticular cell hyperplasia was also seen. Few lymphoid follicles showed diffuse hyperplasia of lymphocytes at eight weeks. At ten weeks, besides diffuse hyperplasia of lymphocytes, a few small microlymphoid follicles were also present.

Many well defined lymphoid follicles with active germinal centres were seen in the spleen of five-week old cyclophosphamide and S. typhimurium treated group. Lymphocytes showed diffuse hyperplasia. At eight weeks also many lymphoid follicles with active germinal centres were seen. Slight reticular cell hyperplasia was also present. Diffuse hyperplasia of lymphocytes, but no lymphoid follicle formation, was seen at ten weeks.

Testosterone treated group

Only a few lymphoid follicles showing diffuse hyperplasia were present at eight weeks in the spleen of testosterone treated uninoculated group. Diffuse hyperplasia of lymphocytes was also seen in ten week old spleen.

There was slight diffuse proliferation of lymphocytes in the spleen of five week old testosterone treated sheep RBC administered group. Focal proliferation of reticular cells was also evident, particularly in periarterial sheath. At

eight weeks, mild diffuse reticular cell hyperplasia was seen and at ten weeks, a few lymphoid follicles showed active germinal centres. Diffuse hyperplasia of lymphocytes and reticular cells was also seen.

Spleen of testosterone and S. typhimurium treated birds showed severe proliferation of lymphoid cells, forming many well defined lymphoid follicles with active germinal centres, at five weeks of age (Plate 10). There was also a slight degree of reticular cell hyperplasia at eight weeks. There were many lymphoid follicles, some of which were hypertrophic. Diffuse hyperplasia of lymphocytes and reticular cells in the periarterial region was also seen (Plate 11). Only a few lymphoid follicles, showing diffuse hyperplasia of lymphocytes, were present at ten weeks.

Antibursal serum treated group

The spleen of antibursal serum treated uninoculated ducklings at ten weeks showed many active lymphoid follicles and germinal centres. There was also diffuse proliferation of lymphocytes and reticular cells.

At five weeks of age in the group dosed with antibursal serum and administered sheep RBC revealed only a few lymphoid follicles with active germinal centres. At ten weeks, there was moderate diffuse hyperplasia of lymphocytes.

A few lymphoid follicles with active germinal centres

Plate 10. Spleen of testosterone treated S. typhimurium inoculated duckling: Proliferation of lymphoid cells. Many well defined lymphoid follicles with active germinal centres.
H & E. X 250

Plate 11. Spleen of testosterone treated S.typhimurium inoculated duckling: Diffuse hyperplasia of lymphocytes and reticular cells in the periarterial region.
H & E. X 250

were present in the spleen of five week old ducklings which were given antibursal serum and S. typhimurium. Proliferation of reticular cells occurred around periarterial sheath at ten weeks of age. There was also formation of microfollicle.

Serum globulins

Ammonium sulphate precipitation of globulins

Ammonium sulphate precipitation of globulins was carried out with pooled serum samples. The protein concentrations of globulins precipitated at 33% and 40% levels of AS0 were 1.688 g % and 5.969 g % respectively.

Sodium sulphate precipitation of globulins

Besides ammonium sulphate, sodium sulphate was also used to precipitate serum globulins. The protein concentration of precipitated globulins was found to be 4 g %.

Gel filtration chromatography

Ammonium sulphate precipitated globulins were concentrated

with polyvinyl pyrrolidone (PVP) and subjected to Sephadex G-200 gel filtration. The typical chromatogram (Fig. 1) revealed two main peaks for the eluted globulin fractions. The elution volume for the first peak was 10 ml and that for the second peak was 20 ml.

The eluted fractions of the ascending limb of the first major peak were pooled and subjected to immunoelectrophoresis using rabbit anti-duck serum. Then a diffuse line extending anodally from the antigen well was obtained (Plate 12). The pooled sample was concentrated with PVP and rerun on the same column to obtain purified IgM.

Fractions of the ascending limb of the second major peak were also pooled and subjected to immunoelectrophoresis. Then a precipitation arc located close to the antigen well was produced, indicating the presence of IgG (Plate 13). As in the case of IgM, this pooled sample was also concentrated with PVP and rerun on the same column to further purify the IgG.

Preparation of antiserum

Antisera to whole serum, globulin and purified IgG and IgM of ducks were raised in rabbits and each antiserum was tested by immunoelectrophoresis using specific antigen.

Immunoelectrophoresis

Immunoelectrophoresis of whole serum of ducks against rabbit antiduck serum produced 13 precipitation arcs, two

FIG 1 SEPHADEX G-200 CHROMATOGRAM OF GLOBULIN

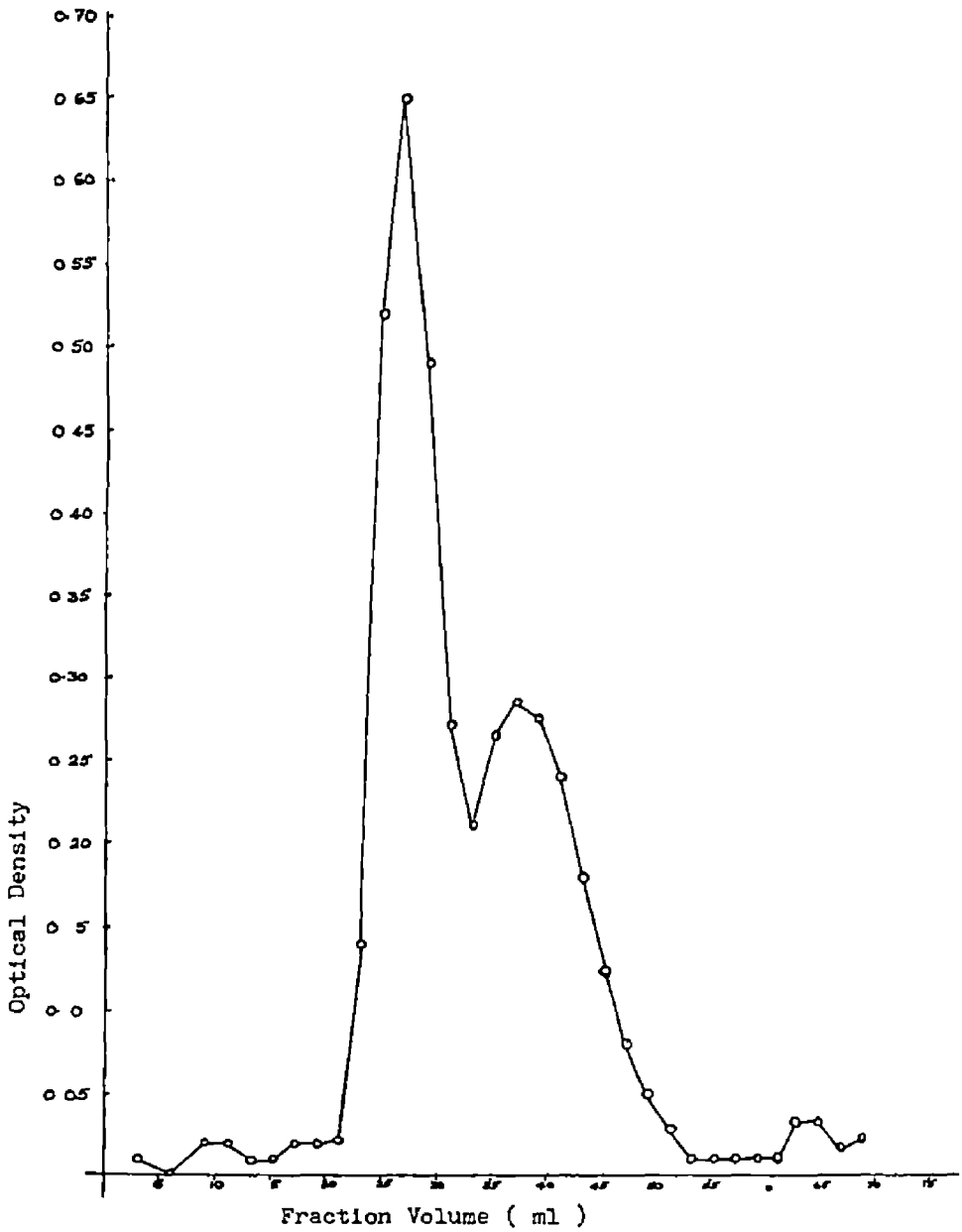


Plate 12. Immunoelectrophoregram of purified duck IgM against specific anti-IgM

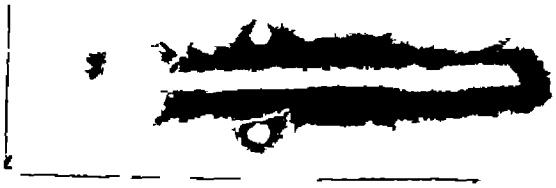
Plate 13. Immunoelectrophoregram of purified duck IgG against specific anti-IgG. Duck IgG upper well and chicken IgG in lower c well

of which corresponded to IgM and IgG arcs (Plate 14). A final concentration of 33% ASS precipitated globulin fraction produced six precipitation arcs against rabbit antiduck serum (Plate 15), while 40% ASS precipitated globulin formed ten arcs of precipitation. Sodium sulphate precipitated globulin on the other hand produced 12 arcs against antiduck serum raised in rabbit. On immunoelectrophoresis of 33% ASS precipitated globulin against rabbit antiduck globulin, two bold precipitation arcs were obtained, one extending from the well anodally and the other seen close to the antiserum though and extending on either side of the antigen well. Besides these, four faint arcs were also seen, extending anodally and these merged with the above two arcs cathodally (Plate 16).

Duck bile on immunoelectrophoresis against antiduck serum raised in rabbit, produced a single precipitation arc, extending anodally from the well (Plate 17).

A precipitation arc extending anodally, directly from the antigen well was produced by immunoelectrophoresis of purified IgM against hyperimmune serum raised in rabbit (Plate 12). Purified IgG produced a precipitation arc close to the antigen well against specific hyperimmune serum (Plate 13). Immunoelectrophoresis of chicken IgG against its anti-IgG revealed that this arc corresponded with that produced by chicken IgG (Plate 18).

Plate 14. Immunoelectrophorogram of duck serum against antiduck serum, showing different arcs of precipitation



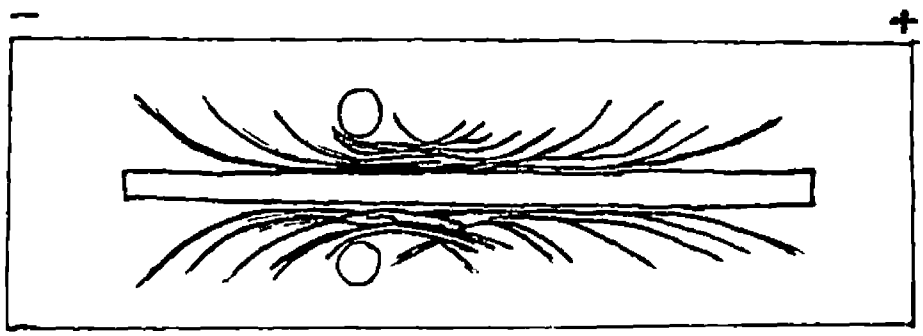


Plate 15. Immunoelectrophorogram of duck globulin against antiduck serum

Plate 16. Immunoelectrophorogram of duck globulin against antiduck globulin

Plate 17. Immunoelectrophorogram of duck bile against anti-duck serum

Plate 18. Immunoelectrophorogram of chicken and duck IgG against anti-chicken IgG

Serum protein

Total serum protein concentration in nonbursectomized and bursectomized ducklings

Pooled serum samples from non-bursectomised (control) and bursectomised ducklings of one to ten weeks of age were assayed for total protein concentration, the values of which are presented in table 12 and graphically represented in fig. 2. Bursectomy was performed by surgical, chemical or hormonal methods, or by using antibursal serum.

It was found that in the non-bursectomised ducklings, the highest level of serum protein (4.313 g %) was at 8th week of age and that the lowest level (1.813 g %) was at the 1st week. The serum protein levels in all other weeks fell within these ranges.

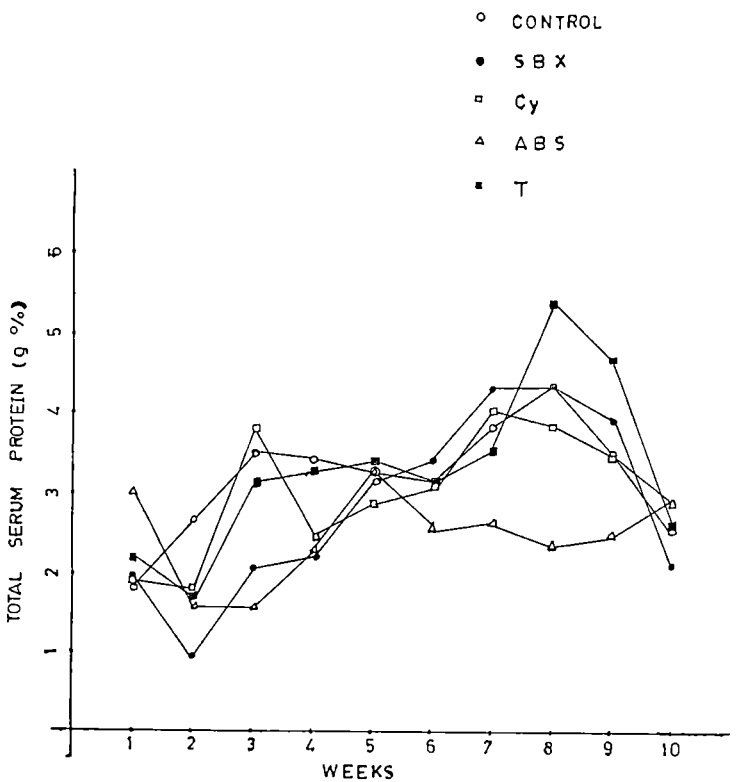
In the surgically bursectomised group of ducklings, the highest serum protein value was found to be 4.313 g %, same as that of the control group, but the maximum serum protein level was observed both at the 7th and 8th weeks of age. The minimum value of 0.9388 g % was recorded in the 2nd week, which was less than the minimum serum protein level observed in the control group.

Cyclophosphamide treated group of ducklings showed a maximum serum protein level of 4.0 g % in the 7th week, which was less than the maximum level for age-matched control group. The minimum level was 1.813 g %, similar to

Table 12. Total serum proteins in non-bursectomised (control) and bursectomised ducklings from 1-10 weeks

Treatments	Total serum proteins (g %) at weeks									
	1	2	3	4	5	6	7	8	9	10
Control	1.813	2.625	3.500	3.375	3.250	3.125	3.830	4.313	3.438	2.500
SBx	1.900	0.938	2.063	2.188	3.125	3.375	4.313	4.313	3.090	2.063
Cy	1.938	1.813	3.813	2.438	2.863	3.063	4.000	3.813	3.420	2.875
T	2.138	1.688	3.125	3.250	3.375	3.125	3.500	5.375	4.625	2.563
ABS	3.000	1.563	1.563	2.313	3.250	2.500	2.625	2.313	2.438	2.875

FIG 2 MEAN TOTAL SERUM PROTEIN CONCENTRATION IN NON-BURSECTOMISED & BURSECTOMISED DUCKLINGS AGED 1-10 WEEKS



that of the control group, but this minimal value was recorded at the 2nd week of age while it was seen in the first week in the control group.

The testosterone treated group showed a maximum serum protein level of 5.375 g % at the 8th week, followed by 4.625 g % in the 9th week. The minimum value (1.688 g %) seen in the second week of age was less than that in the control group.

In the antibursal serum treated group, the maximum serum protein level (3.25 g %) was observed at 5 weeks of age, which was less than the maximum level for control and other treatment groups. The minimum level was found to be 1.563 g %, at 2nd and 3rd weeks of age, which was also less than that of the control.

Among the bursectomised groups, the maximum level of serum protein was observed in the testosterone treated group (5.373 g %) and the minimum level (0.938 g %) in the surgically bursectomised group (Fig. 2). The maximum serum protein level was observed at the 8th week of age in the control, surgically bursectomised and testosterone treated groups, whereas in the Cy treated and ABS treated groups, the maximum levels were seen at the 7th and 5th weeks respectively. From the table 12, it could be seen that the serum protein levels at 10th week showed a decline in all the groups except ABS group.

Total serum protein in non-bursectomised and bursectomised ducklings inoculated with SRBC/S. typhimurium

Mean total serum protein values in non-bursectomised and bursectomised ducklings after inoculation with SRBC/ S. typhimurium are given in table 13 and Fig. 3-6. The control birds in this case were non-bursectomised ducklings inoculated with either SRBC (CSR) or S. typhimurium (CST) Bursectomised ducklings formed the treatment group in this experiment. Bx was performed by any one of the four methods - surgical (Sx), chemical (Cy), hormonal (T) or by administration of antibursal serum (ABS). Similar to the controls, the bursectomised groups were also inoculated with SRBC or S. typhimurium. The inoculations were done on the 7th (group I), 28th (group II) or 42nd (group III) days of age and the serum protein values were determined on 7, 14, 21 and 28 days post-inoculation, in each group.

Seventh day post-inoculation

The highest value of mean total serum protein concentration of CSR ducklings was seen in group II (4.313 g %) and the lowest in group III (3.0 g %), while in group I, an intermediary level (3.375 g %) was seen. In CST ducklings, maximum serum protein value was observed in group I (4.0 g %), followed by group II (3.75 g %) and group III (3.625 g %) (Table 13).

Among the bursectomised ducklings, the highest concentration of serum proteins (6.688 g %) was noticed in group III

Table 13. Total serum protein (g %) in non-bursectomised and bursectomised ducklings of 3 age groups inoculated with SRBC/*S. typhimurium*

Treatment	Group I				Group II				Group III			
Days after inoculation	7	14	21	28	7	14	21	28	7	14	21	28
CSR	3.375	3.125	3.375	4.063	4.313	4.313	3.813	4.313	3.000	3.000	2.750	4.813
CSt	4.000	3.813	3.375	4.688	3.750	3.125	4.188	4.688	3.625	6.500	3.375	4.063
SBxSR	2.188	2.625	2.625	3.625	3.375	3.625	3.625	3.625	2.875	2.875	3.265	3.140
SBxSt	1.938	2.438	3.075	3.125	3.250	4.813	3.813	4.313	3.375	2.438	2.188	3.375
CyCR	2.930	3.813	5.250	4.313	4.063	5.250	6.500	3.375	6.375	4.000	4.125	4.270
CySt	3.525	4.835	7.188	4.563	3.625	4.313	3.125	3.125	6.688	6.000	6.250	6.250
TCR	2.875	3.125	3.240	3.375	3.250	3.375	4.000	3.845	7.250	2.188	3.813	3.916
TSt	3.375	2.875	2.438	3.813	3.625	2.875	4.438	4.438	3.625	2.438	3.125	2.875
ABCR	3.750	2.500	2.875	4.963	2.875	4.813	3.313	3.813	3.125	3.625	3.375	3.625
ABSt	2.875	3.125	4.063	4.563	6.688	4.813	4.813	4.213	4.313	2.188	4.063	5.000

Group I - Antigen inoculated at day 7
Group II - Antigen inoculated at day 28
Group III- Antigen inoculated at day 42

FIG 3 HISTOGRAM REPRESENTING MEAN TOTAL SERUM PROTEIN CONCENTRATION IN NON BURSECTOMISED AND BURSECTOMISED DUCKLINGS, 7DAYS AFTER INOCULATION WITH S R B C / S typhimurium

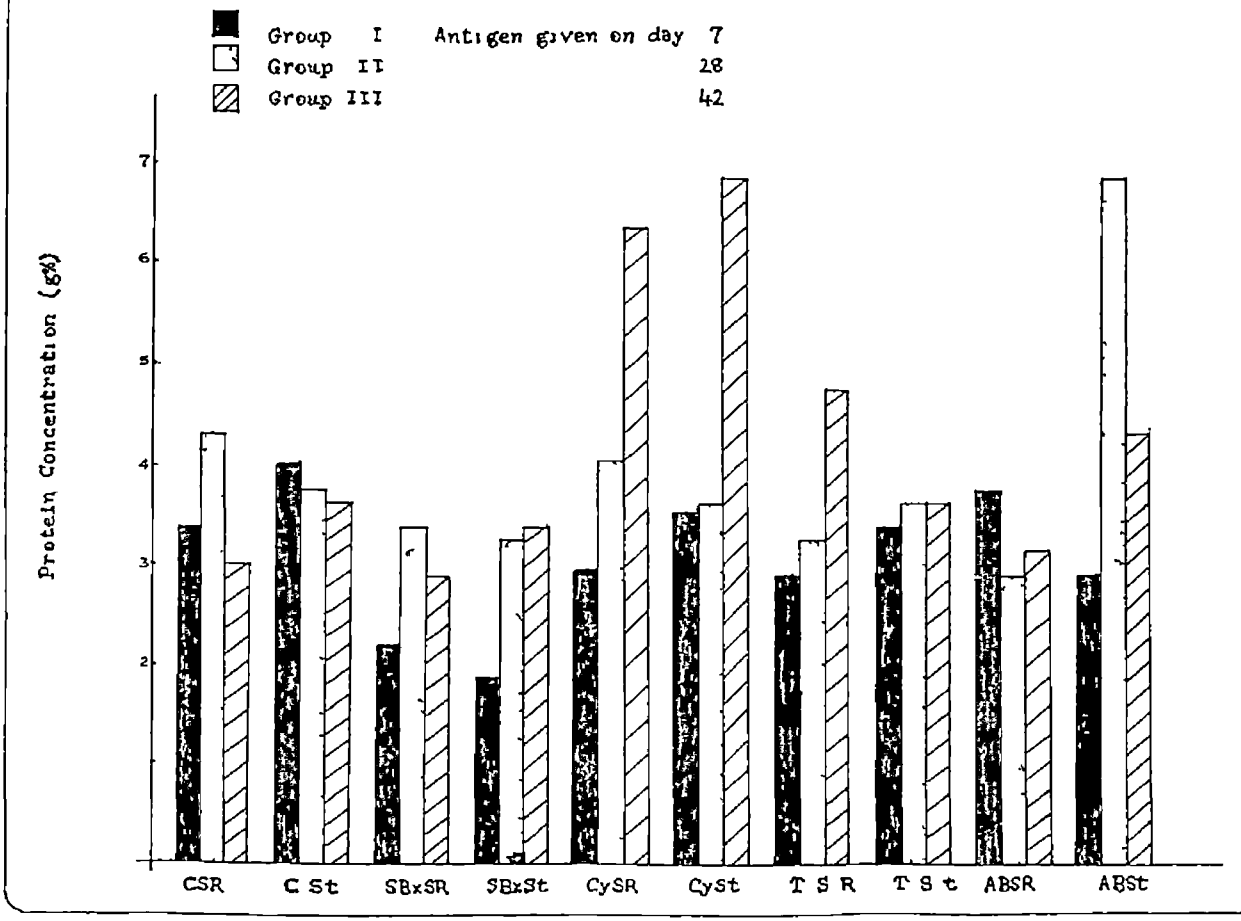


FIG 4 HISTOGRAM REPRESENTING MEAN TOTAL SERUM PROTEIN CONCENTRATION IN NON BURSECTOMISED AND BURSECTOMISED DUCKLINGS , 14 DAYS AFTER INOCULATION WITH S R B C / S typhimurium

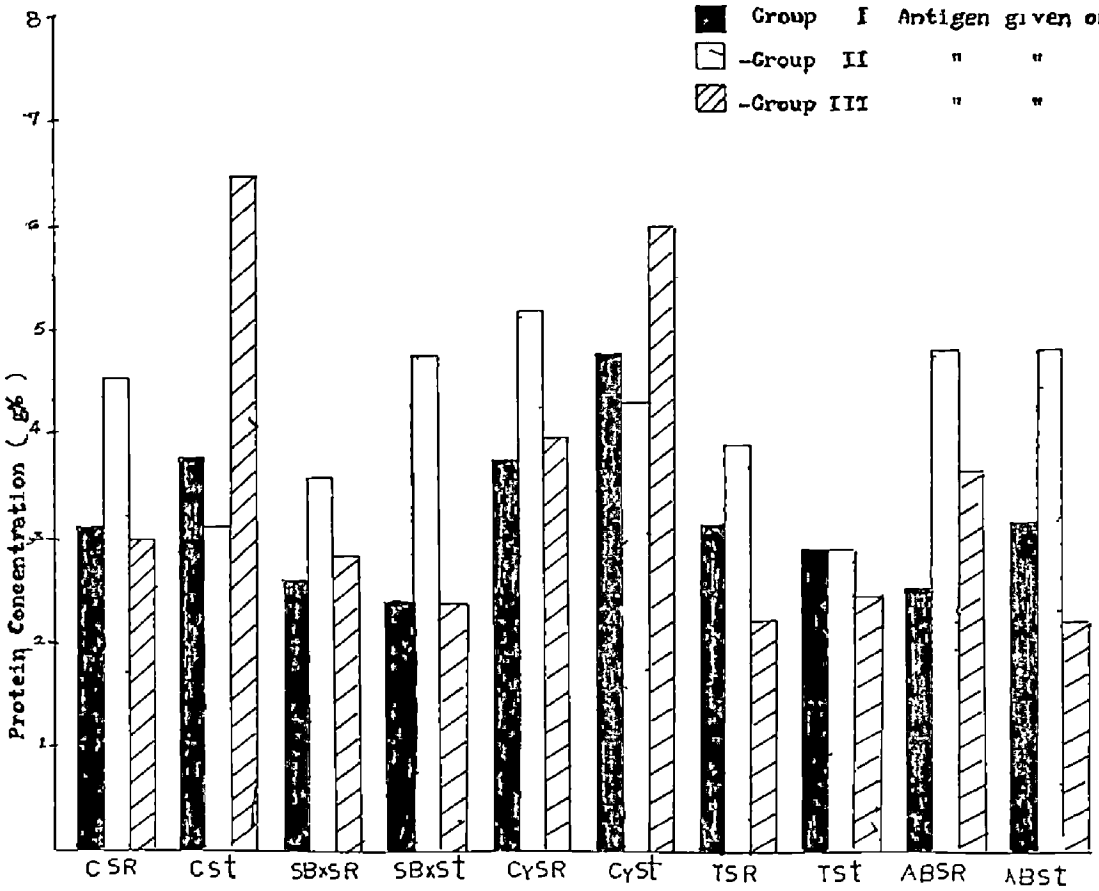


FIG 5 HISTOGRAM REPRESENTING MEAN TOTAL SERUM PROTEIN CONCENTRATION IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS, 21 DAYS AFTER INOCULATION WITH SRBC/S typhimurium

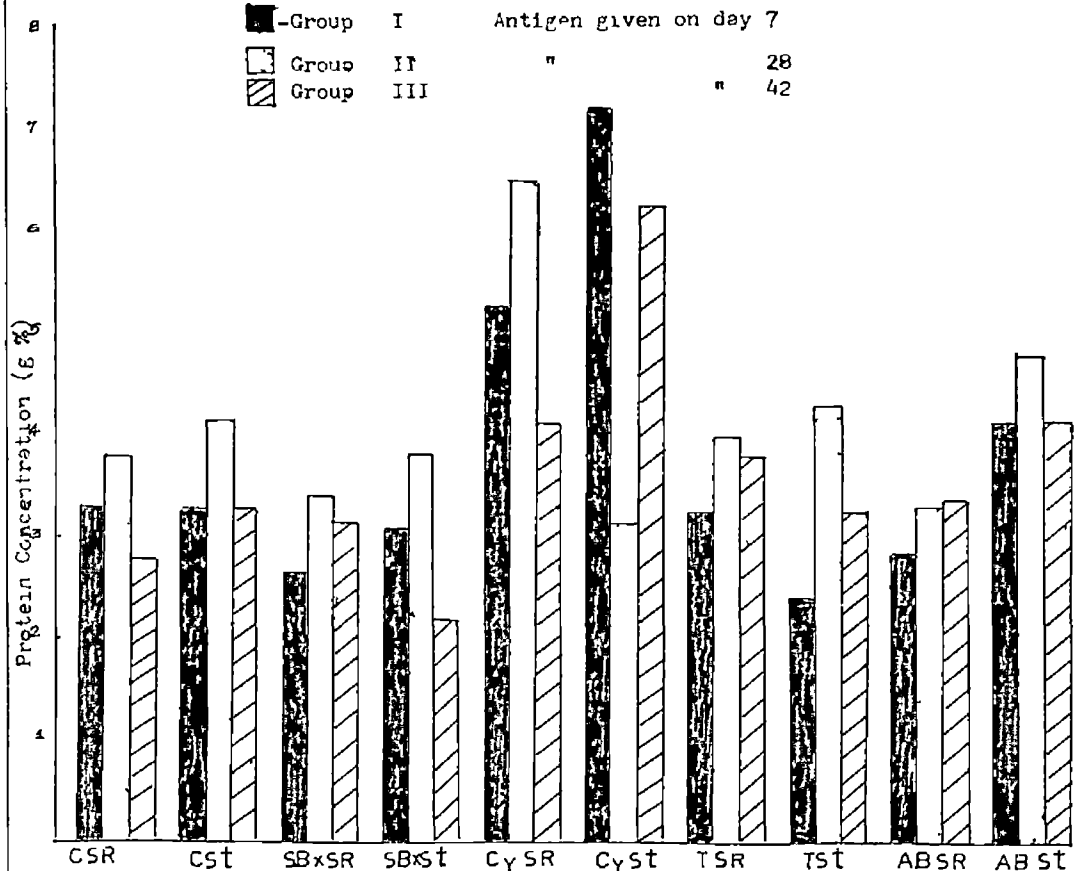
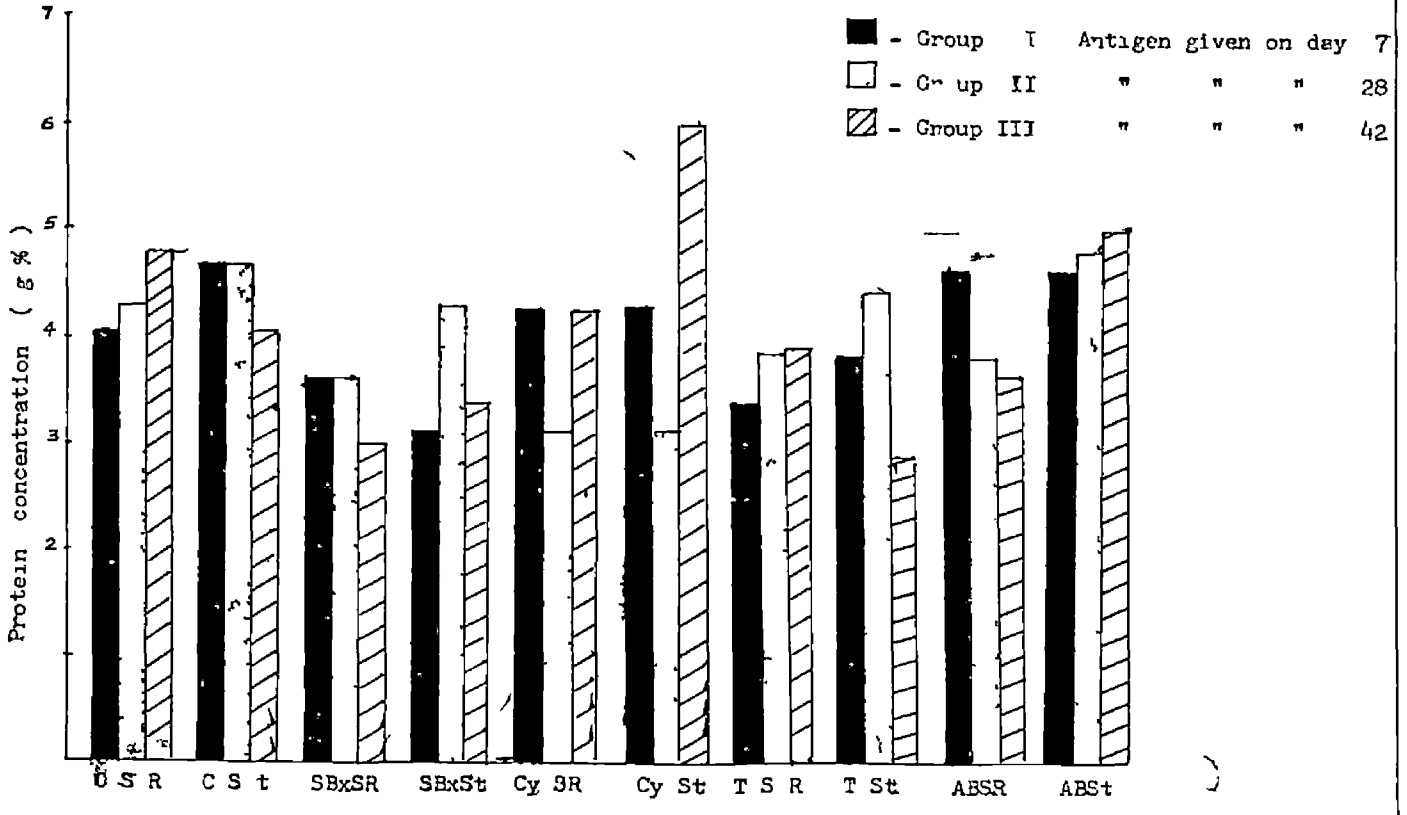


FIG 6 HISTOGRAM REPRESENTING MEAN TOTAL SERUM PROTEIN CONCENTRATION IN NON-BURSECTOMISED AND BURSECTOMISED DUCKLINGS , 28 DAYS AFTER INOCULATION WITH S R B C / S typhimurium



of CySt and group II of ABST and the lowest value in group I of SBxSt (1.938 g %) (Fig.3).

In group I bursectomised ducklings administered SRBC, the highest level of mean serum protein was 3.750 g %, in ABSR and the lowest level was 2.188 g %, in SB x SR. Compared to CSR group I, only ABSR showed higher protein level while SBxSR, CySR and TSR showed lower levels. In group II, the mean total serum protein concentration ranged from 4.063 g % (CySR) to 2.875 g % (ABSR). But in comparison with CSR group II, all the bursectomised ducklings given SRBC showed lower serum total protein values in group II. CySR revealed highest level of protein in group III (6.375 g%), while the lowest level was seen in SBxSR (2.875 g %). Only SBxSR had lower protein level compared to CSR group III, while CySR, TSR and ABSR showed higher levels. Comparing group I to III, group III ducklings showed highest levels of serum proteins in CySR and TSR, while in SBxSR, group II and in ABSR, group I, showed highest levels (Fig.3).

Salmonella typhimurium inoculated bursectomised ducklings of group I revealed highest serum total protein level in CySt (3.525 g %) and lowest level in SBxSt (1.933 g %). On comparison with CSt group I, all the bursectomised ducklings had lower serum protein values. Group II bursectomised ducklings showed a serum protein level ranging from 6.688 g % (ABSt) to 3.250 g % (SBxSt). Compared to CSR group II, only

ABSt had higher protein levels, while SBxSt, CySt and TSt had lower levels (Fig.3). In group III, S. typhimurium inoculated bursectomised ducklings showed highest level of serum proteins (6.688 g %) in CySt and the lowest level (3.375 g %) in SBxSt. In this case, higher serum protein levels than that of CSt group III were observed in CySt and ABSt, while TSt showed comparable level and in SBxSt the level was lower (Fig. 3).

A comparison of the three groups in S. typhimurium inoculated and bursectomised ducklings revealed that group III had highest levels of serum proteins in CySt and SBxSt, while in ABSt, group II had highest level. In TSt, both groups II and III showed identical high levels, which were greater than that of group I (Fig. 3).

Fourteenth day post-inoculation

On the 14th day post-inoculation, the mean total serum protein of CSR group showed the highest value (4.313 g %) in group II, and the lowest value (3.0 g %) in group III. Group I showed an intermediary level of 3.125 g %. In CSt ducklings, maximum serum protein levels were observed in group III (6.500 g %), followed by group I (3.813 g %) and group II (3.125 g %) (Fig. 4).

Among the bursectomised ducklings, the highest concentration of total serum protein was noted in group III of CySt (6.0 g %) and the lowest value (2.188 g %) in group III TSR and ABSt.

In SRBC inoculated bursectomised ducklings of group I, the mean serum protein values from highest to lowest were in the following order: CySR (3.813 g %), TSR (3.125 g %), SBxSR (2.625 g %) and ABSR (2.50 g %). Of these the value of CySR was higher than that of CSR group I, while that of TSR was similar to the control. In group II, CySR showed a maximum serum protein level of 5.25 g %, followed by ABSR (4.813 g %). SBxSR were higher than that of the control ducklings of the same group. Group III bursectomised ducklings had a maximum serum protein concentration in CySR (4.0 g %), followed by ABSR (3.625 g %). These values were greater than that of the control (3.0 g %). Comparing all the three groups inoculated with SRBC, group II showed highest values in all the bursectomised birds, followed by group III in all cases except TSR, where group I value was higher than group III values (Table 13 and Fig.4).

Bursectomised and S. typhimurium inoculated ducklings of group I revealed a serum protein value higher than that of CSt only in CySt (4.095 g %), while the lowest value was seen in SBxSt (2.438 g %). In group II ducklings, higher serum protein concentration than CSt group II was observed in SBxSt (4.813 g %), ABSt (4.813 g %) and CySt (4.313 g %). TSt recorded a lower value (2.875 g %) than control. All the treated ducklings coming under group III showed lower serum protein values compared to CSt of same group. Among the group III treated birds, the highest serum protein value

was seen in CySt (6.0 g %) and the lowest in ABSt (2.188 g%).

Comparing the serum protein values of the three groups treated with S. typhimurium a higher value for group II was found in SBxSt and ABSt, while the protein value of group III was higher in CySt and in TSt, both group I and II values were identical.

Twenty-first day post-inoculation

On the twenty-first day post-inoculation, the mean total serum protein of CSR group II showed highest value of 3.813 g %, followed by group I (3.375 g %) and group III (2.750 g %). In CSt ducklings also, the maximum total serum protein was in group II (4.188 g %), whereas the groups I and III showed same values (3.375 g %) (Table 13).

Among the bursectomised ducklings, the highest concentration of mean total serum protein was noted in group I of CySt (7.188 g %) and lowest value in group III SBxSt (2.188 g %).

In SRSC inoculated bursectomised birds, group I showed total serum protein concentration in the range of 5.250 g % to 2.625 g %, the highest value being in CySR and the lowest in SBxSR. Among the treated birds, only CySR had a value higher than that of CSR group I. The range of total serum protein for group II was from 6.500 g % (in CySR) to 3.313 g % (in ABSR). In the treated birds of this group, only CySR and TSR showed values higher than that of CSR

group II. The highest total serum protein level in group III was shown by CySR (4.125 g %) and the lowest by SBxSR (3.265 g %). All the four treatment groups of SBxSR, CySR, TSR and ABSR under group III had higher serum protein values, compared to CSR group III.

Comparing all the three groups inoculated with SRBC, group II showed highest serum protein values in SBxSR, CySR and TSR, followed by group II in SBxSR and TSR. In ABJR, the highest value was shown by group III, while in CySR, group III had the lowest value (Fig. 5).

Among S. typhimurium inoculated birds of group I, the highest total serum protein value was shown by CySt (7.188 g%) and the lowest by TSt (2.438 g %). Of the different treatments in group I, CySt and ABSt showed higher protein values than CSt group I. In group II, the serum protein values ranged from 4.813 g % (in ABSt) to 3.125 g % (in CySt). Compared to CSt group II, only TSt and ABSt had higher values (Fig. 5). The serum protein range in group III was from 6.250 g % (in CySt) to 2.188 g % (in SBxSt). CySt and ABSt ducklings showed greater mean serum protein levels compared to the CSt group III.

In the case of S. typhimurium inoculated bursectomised ducklings, group II revealed higher serum protein levels in ABSt, TSt and SBxSt, compared to groups I and III. In CySt ducklings, group II value was the lowest and group I value

the highest. In ABSt, groups I and III revealed the same values of 4.063 g % (Fig. 5).

Twenty-eighth day post-inoculation

The highest mean serum protein value in CST on the 28th day post-inoculation was recorded in group III (4.813 g %), and the lowest in group I (4.063 g %). Group II of CSR recorded an intermediary value of 4.313 g %. In CSt, groups I and II showed high total protein values of 4.688 g% and in group III the value was 4.063 g%, similar to CST group I (Table 13).

In bursectomised ducklings inoculated with SRBC/S.typhimurium, highest concentration of total serum protein was observed in group III of CySt (6.250 g %) and the lowest in TSt group III (2.875 g %).

Among the bursectomised ducklings inoculated with SRBC, the total serum protein values of group I were in the range of 4.563 g % to 3.375 g %, the highest value being in ABSR and the lowest in TSR. Compared to CSR group I, CySR and ABSR showed higher serum protein values. In group II, the total serum protein level ranged from 3.845 g % (in TSR) to 3.375 g % (in CySR). All the four treatments in group II showed lesser protein values than CSR group II. Group III of SRBC inoculated bursectomised ducklings recorded a serum protein range of 4.270 g % (in CySR) to 4.140 g % (in SBxSR). Similar to group II, in group III also all the four treatments

revealed lower levels of serum protein, compared to CSR group III.

On comparing the three groups of bursectomised ducklings given SRBC, group I of ABSR and CySR recorded higher serum protein values than other groups, while in SBxSR, groups I and III showed equal values. In TSR, group III was having higher protein level compared to the other two groups (Fig.6).

In the S. typhimurium inoculated bursectomised birds, the highest value of total serum protein in group I was seen in ABSt (4.563 g %) and the lowest value in SBxSt (3.125 g %). Compared to CSt group I, all the treatments under bursectomised group I had lower protein values. In group II, the highest total serum protein level was recorded in ABSt (4.813 g %) and the lowest in CySt (3.125 g%). Only ABSt showed higher protein value compared to CSt group II. The mean serum protein level in group III ranged from 6.250 g % (in CySt) to 2.875 g % (in TSt). Only CySt and ABSt had higher protein values, compared to CSt group III.

A comparison between the three groups of S. typhimurium inoculated birds revealed that group III had higher protein values in CySt and ABSt, while group II showed high values in SBxSt and TSt (Fig.6).

Serological tests

a) Bacterial agglutination to detect antibody against S. typhimurium

S. typhimurium antigen was inoculated to non-bursectomised

(control) and bursectomised ducklings on the seventh (group I), 28th (group II) or 42nd (group III) days of age and the agglutination titres were determined from pooled serum samples, on days 7, 14, 21 and 28, post-inoculation. The results are given in table 14 and represented by histograms in figs.7-10. The titres were expressed as the reciprocal of the highest dilution giving complete agglutination.

Seventh day post-inoculation

On seventh day post-inoculation, the mean antibody titre in control group I was the highest (1024) followed by the group II and III (512 each) (Table 14).

Among the bursectomised ducklings in SBx birds, only group II had any antibody titre (32), while no titre was observed in groups I and III.

In Cy treated ducklings, group I titre was higher (32) compared to group II titre (8), while group III did not give any antibody titre.

Testosterone treated ducklings showed the highest antibody titre of 32 in group III, followed by a lower titre of 8 in group II, while group I failed to show any titre.

Antibursal serum administered birds gave an antibody titre of 64 in group II only, while no titre was obtained for both groups I and III (Fig. 7).

The highest antibody titre among the bursectomised

Table 14. Antibody titre* in non-bursectomised and bursectomised ducklings inoculated with *S. typhimurium*

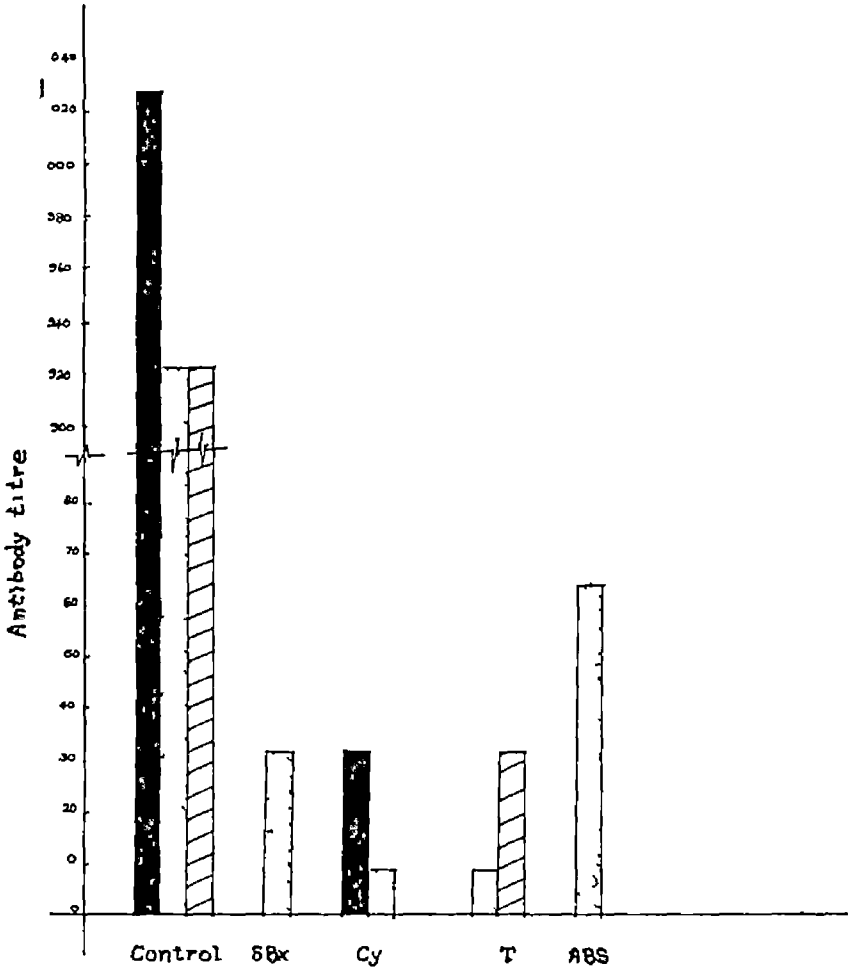
Treatment	Group I				Group II				Group III			
Days after inoculation	7	14	21	28	7	14	21	28	7	14	21	28
Control	1024	2048	1024	1024	512	1024	1024	1024	512	1024	1024	2048
SBx	-	64	128	128	32	32	64	32	-	8	32	32
Cy	32	32	-	-	8	128	32	-	-	-	-	-
T	-	256	256	64	8	64	32	32	32	-	32	128
ABS	-	128	128	128	64	128	128	64	-	128	64	8

* The titres are expressed as the reciprocal of the highest dilution showing complete agglutination

Group I - Antigen given on day 7
Group II - Antigen given on day 28
Group III - Antigen given on day 42

FIG 7 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS, 7 DAYS AFTER INOCULATION WITH *S typhimurium*

■	Group	I	Antigen given at day 7
□	Group	II	28
▨	Group	III	42



ducklings was in group II ABS and the lowest in group II Cy and testosterone treated birds. All these titre values were very low, compared to the values obtained for all the three groups of the control.

Fourteenth day post-inoculation

In non-bursectomised birds, on the 14th day post-inoculation, group I revealed maximum antibody titre of 2048, while groups II and III showed the same titre of 1024 (Table 14).

Among the bursectomised birds, the highest titre (256) was shown by group I of testosterone given ducklings and the lowest titre (8) by group III of SBx ducklings.

In SBx ducklings, group I recorded the maximum titre (64), followed by group II (32) and group III (8) (Fig.8).

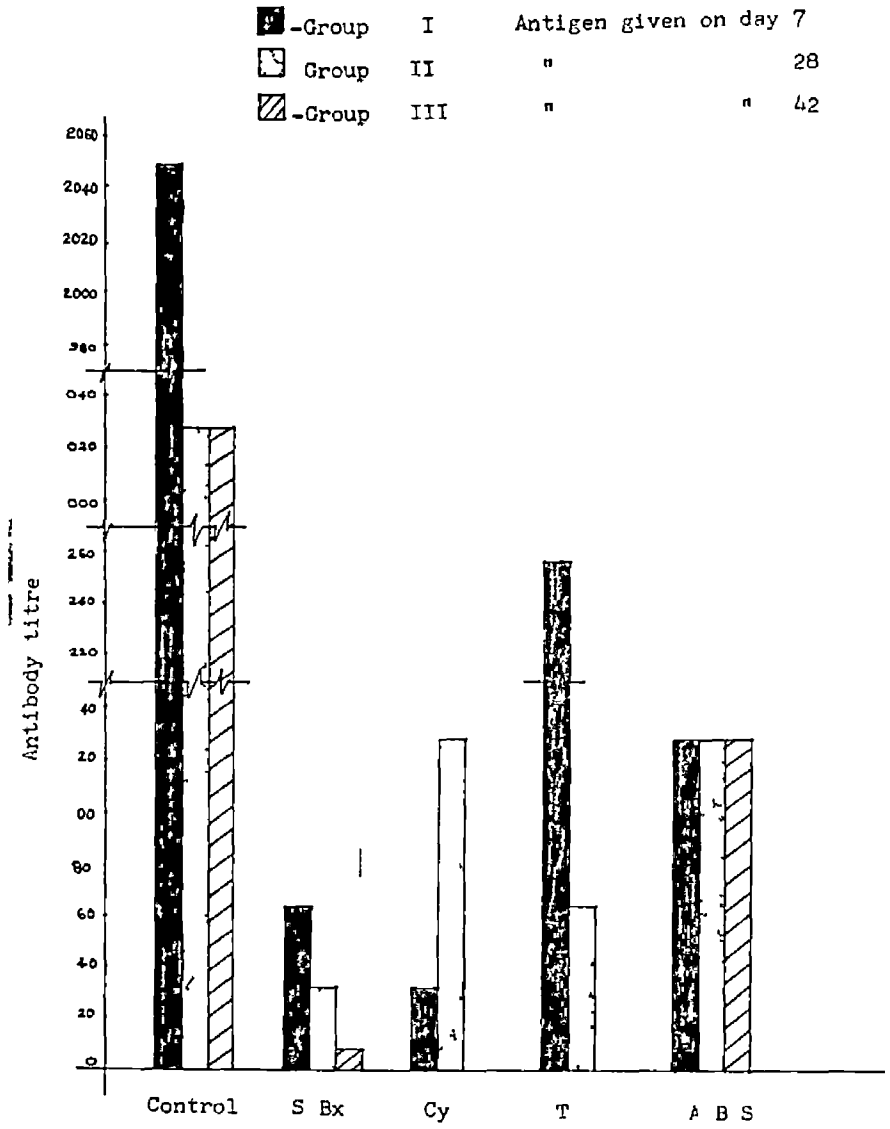
Cyclophosphamide treated ducklings showed the highest titre of 128 in group II and the lowest of 32 in group I, while group III failed to give any titre.

In testosterone treated birds group I gave the highest titre of 256, followed by group II (64) and no titre was observed in group III.

Antibursal serum administered ducklings showed the same titre of 128 in all these groups.

As in the previous case, here also all the bursectomised ducklings showed very low titres, compared to the controls.

FIG 8 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS 14 DAYS AFTER INOCULATION WITH S typhimurium



Twentyfirst day post-inoculation

In non-bursectomised ducklings, all the three groups showed the same titre of 1024 (Table 14).

Among the bursectomised birds, the highest titre (256) was in group I of testosterone treated ducklings and the lowest titre of 32 was seen in SBx group III, Cy group II and testosterone groups II and III.

In SBx ducklings, the maximum antibody titre was 128 in group I, followed by 64 in group II and 32 in group III.

Cyclophosphamide treated birds showed a titre of 32 in group II while both groups I and III did not give any titre.

In testosterone given ducklings, the highest titre (256) was in group I, while the groups II and III shared the same titre of 32.

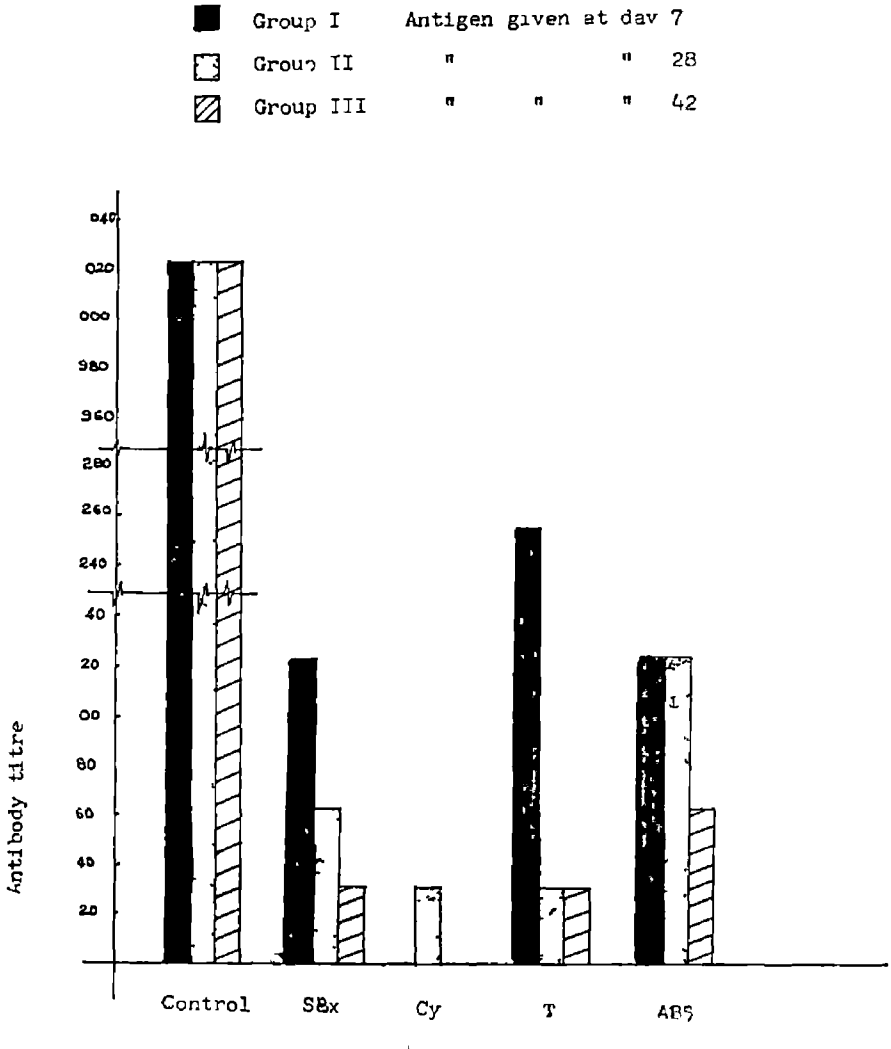
Antibursal serum administered ducklings revealed identical titres of 128 in groups I and II, while group III showed a lower titre of 64.

On 21 days post-inoculation also the titres of bursectomised birds were far below that obtained in control ducklings (Fig. 9).

Twenty-eighth day post-inoculation

Non-bursectomised ducklings on 28th day post-inoculation

FIG 9 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS, 21 DAYS AFTER INOCULATION WITH S typhimurium



revealed the same antibody titres of 1024 in groups I and II, while group III had a titre of 2048 (Table 14).

Group I of SBx and ABS treated and group III of testosterone given ducklings recorded the highest titres of 128, while group III of ABS had the lowest titre of 8.

In SBx ducklings, group I had the maximum titre of 128, while identical titres of 32 were given by groups II and III.

Cyclophosphamide treated birds did not show any antibody titre in groups I, II and III (Fig.10).

Testosterone administered ducklings showed the highest titre of 128 in group III, followed by group I (64) and group II (32).

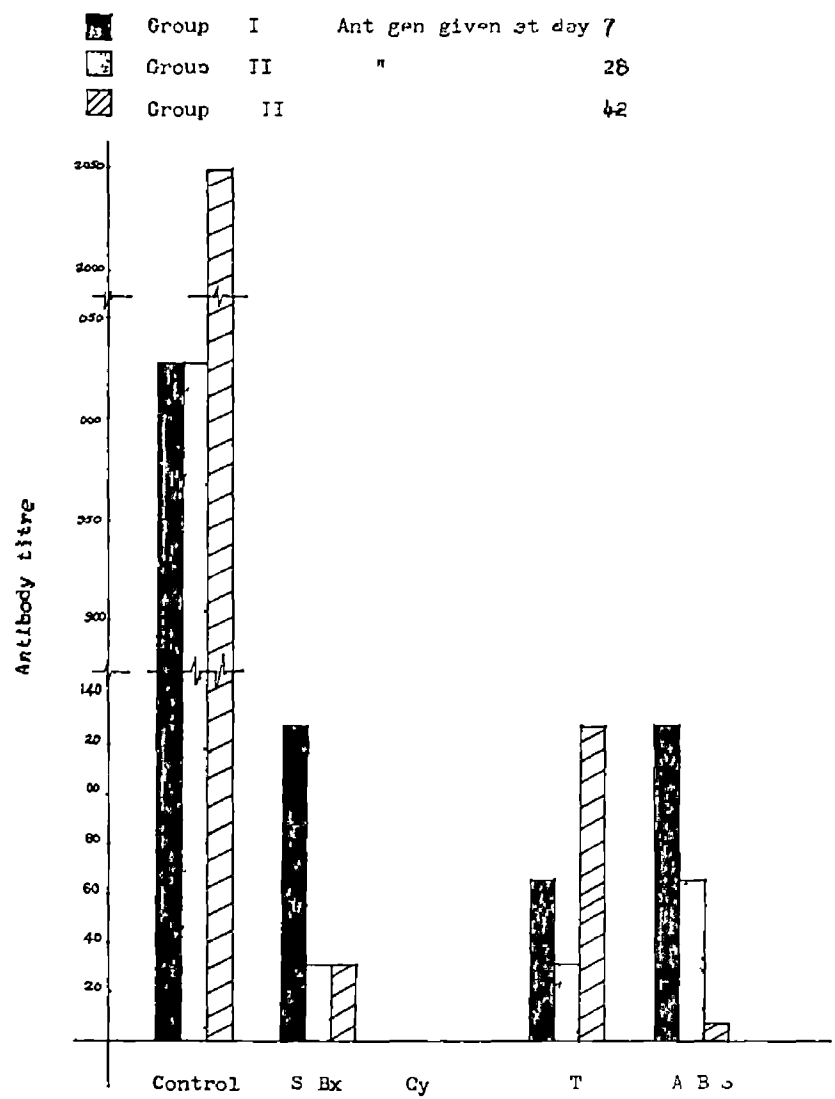
In ducklings given antibursal serum, the antibody titres were in the following order: group I (128), group II (64) and group III (8).

Compared to the control groups, ducklings of all the bursectomised groups had very low antibody titres.

b) Sheep red blood cell agglutination to detect hemolysin

Antibody titres in SRBC inoculated non-bursectomised and bursectomised ducklings were determined from pooled serum samples, on 7, 14, 21 and 28 days post-inoculation. The antigen was inoculated at day 7 (group I), day 28 (group II) or day 42 (group III). The results obtained

FIG 10 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS, 28 DAYS AFTER INOCULATION WITH S typhimurium



are presented in table 15 and represented by histograms in figs. 11-14. The titres were expressed as the reciprocal of the highest dilution giving complete agglutination.

Seventh day post-inoculation

On seventh day post-inoculation, the highest antibody titre against SRBC in non-bursectomised ducklings was in group III (512), followed by group II (256) and group I (128).

Among the bursectomised ducklings, group III of testosterone treated and group I of AB3 treated ducklings had the highest titre (128), while the lowest titre (8) was seen in group III of Cy and group I of testosterone treated birds.

In BPH ducklings, group III had highest titre of 64, followed by group I (32) and group II (16).

Cyclophosphamide treated ducklings revealed a maximum antibody titre of 32 in group II and a minimum titre of 8 in group III. Group I showed an intermediary titre of 16.

The antibody titres given by testosterone given birds were in the following order: group III (128), group II (32) and group I (8).

Antibursal serum administered ducklings recorded the maximum titre of 128 in group I, while group II and III shared the same titre of 32.

Table 15. Antibody titre* in non-bursectomised and bursectomised ducklings inoculated with SRBC

Treatment	Group I				Group II				Group III			
Days after inoculation	7	14	21	28	7	14	21	28	7	14	21	28
Control	128	256	256	512	256	512	512	512	512	256	256	256
SBx	32	32	32	64	16	16	32	64	64	64	64	64
Cy	16	32	16	8	32	128	32	8	8	-	-	-
T	8	64	128	128	32	64	128	128	128	64	64	16
ABS	128	256	256	256	32	16	16	16	32	32	32	128

* The titres are expressed as the reciprocal of the highest dilution showing complete agglutination

Group I - Antigen given at day 7

Group II - Antigen given at day 28

Group III - Antigen given at day 42

FIG 11 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS, 7 DAYS AFTER INOCULATION WITH SRBC

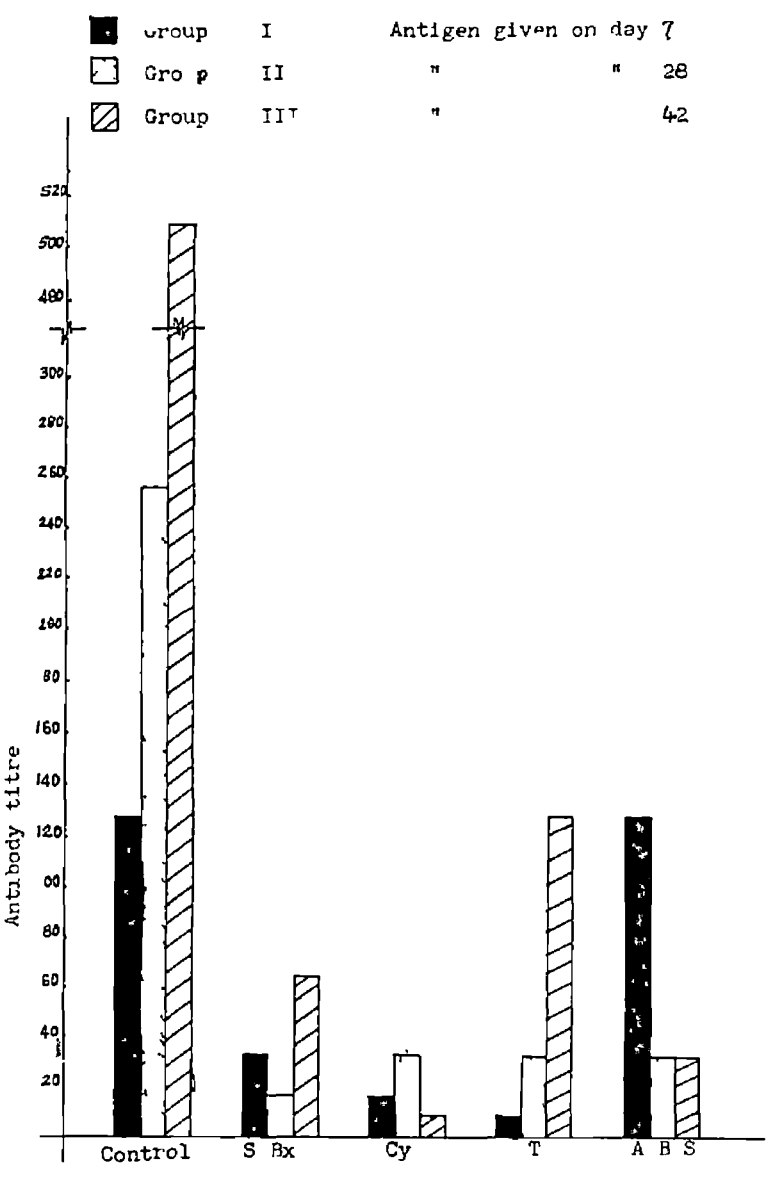


FIG 12 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS 14 DAYS AFTER INOCULATION WITH SRBC

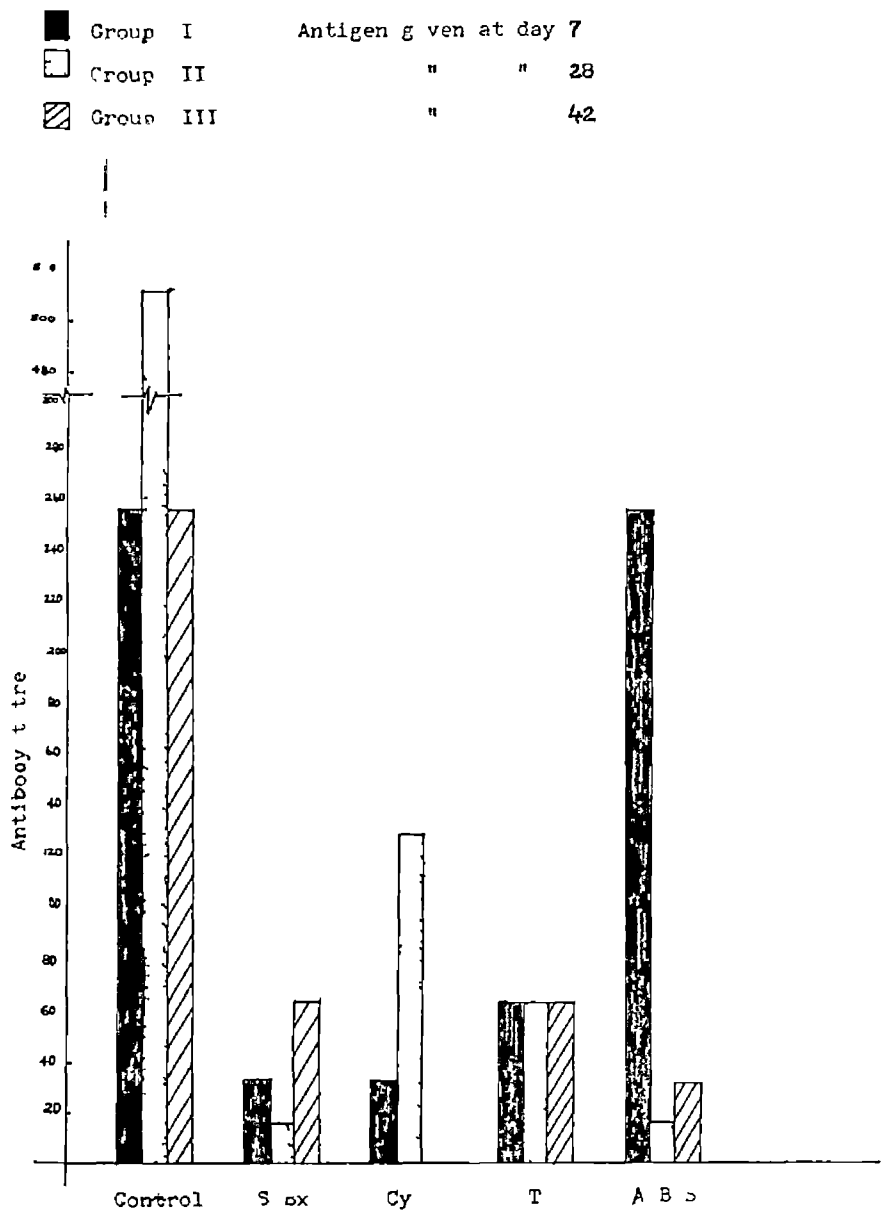


FIG 13 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS, 21 DAYS AFTER INOCULATION WITH SRBC

Group I Antigen given at day 7
Group II " " " 28
Group III " 42

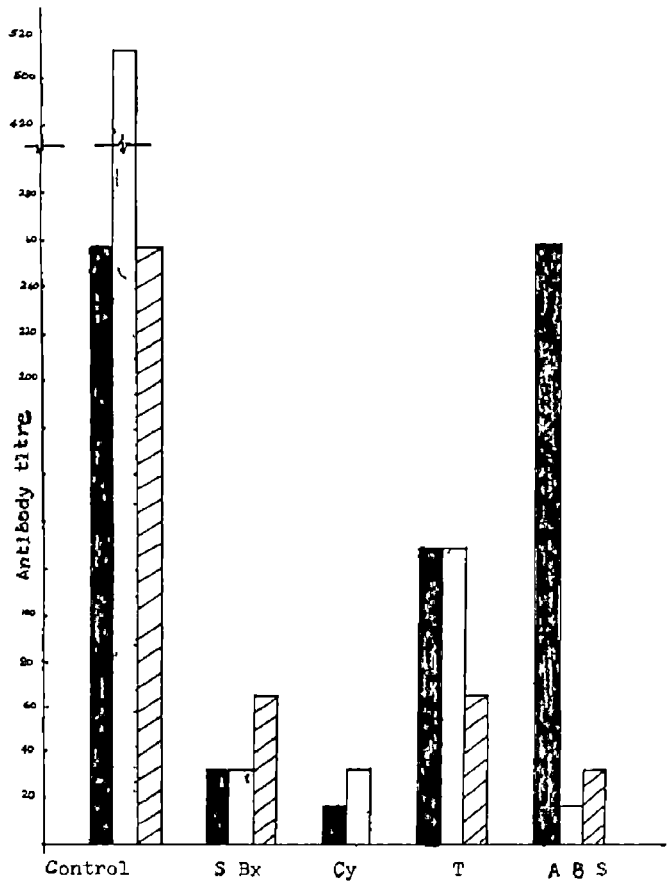
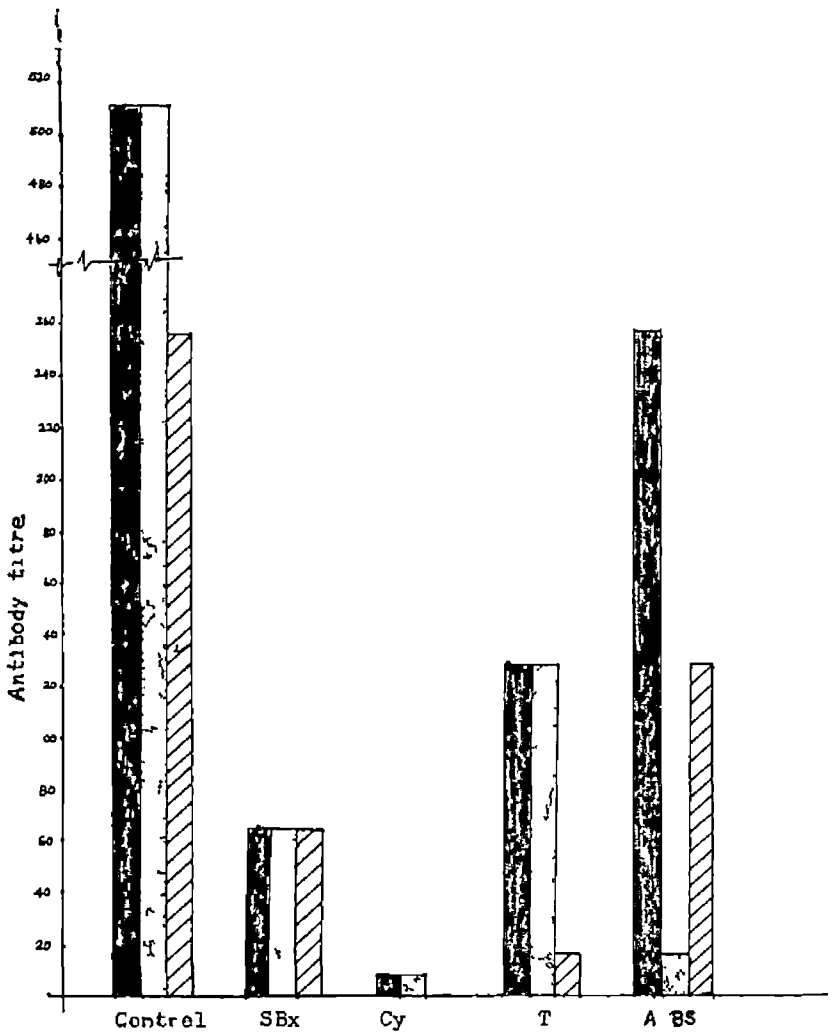


FIG 14 HISTOGRAM REPRESENTING MEAN ANTIBODY TITRE IN NON-BURSECTOMISED & BURSECTOMISED DUCKLINGS, 28 DAYS AFTER INOCULATION WITH S R B C

Group I Antigen given at day 7
Group II " " 28
Group III " " 42



On comparing with the control groups I to III, all the bursectomised ducklings revealed very low antibody titres against SRBC (Fig.11).

Fourteenth day post-inoculation

The non-bursectomised ducklings at 14th day post-inoculation revealed anti-SRBC titres in the following order: group II (512) and groups I and III (256).

In bursectomised ducklings, the maximum titre was given by ABS group I (256) and the minimum by group II of SBx and ABS (16).

Among SBx ducklings, group III had maximum titre of 64, followed by group I (32) and group II (16).

Cyclophosphamide given ducklings showed the maximum titre of 128 in group II and the minimum of 32 in group I, while no titre was obtained in group III.

Neostatenone treated ducklings revealed the same titre (64) in all the three groups.

Antibursal serum administered ducklings recorded a maximum titre of 256 in group I, followed by 32 in group III and 16 in group II.

In comparison with the control groups, only group I of ABS showed the same antibody titre as groups I and III of control, while all the other treatments had very low titres (Fig. 12).

Twentyfirst day post-inoculation

On 21st day post-inoculation, non-bursectomised ducklings revealed maximum antibody titre in group II (512), while groups I and III had the same titre of 256.

Among the bursectomised birds, the maximum antibody level was in group I of ABS (256) and the minimum (16) in group I of Cy and group II of ABS treated ducklings.

Surgically bursectomised ducklings showed highest antibody titre (64) in group III, while groups I and II shared the same titre of 32.

In cyclophosphamide treated ducklings, the maximum antibody titre of 32 was in group II and the minimum of 16 in group I, while group III failed to give any titre.

Testosterone administered ducklings showed similar titres of 128 in groups I and II, whereas group III showed a lower titre of 64.

In ABS treated ducklings, the highest antibody titre of 256 was in group I, while lower titres of 32 and 16 were recorded in groups III and II respectively.

As in the case of 14 days post-inoculation, only ABS group I had comparable titres with group I and III of control, while all the rest had lower titres (Fig. 13).

Twenty-eighth day post-inoculation

Non-bursectomised ducklings at 28th day post-inoculation

revealed comparable maximum antibody titres of 512 in groups I and II, while group III had a lower titre of 256.

Among the bursectomised ducklings, group I of ABS treatment had the maximum titre of 256, while groups I and II of Cy treatment had the minimum titre of 8.

In surgically bursectomised ducklings, antibody titres in all three groups were the same (64).

Cyclophosphamide administered birds showed very low antibody titre of 8 in groups I and II, whereas group III did not give any titre.

Testosterone treated ducklings recorded a maximum antibody titre of 128 in groups I and II, while group III had a very low titre of 16.

Antibursal serum given ducklings had the highest level of antibody (256) in group I, followed by 128 in group III and 16 in group II.

When compared with the control groups, only ABS treated group I had identical titre with control group III, while all others had lower titres (Fig.14).

Quantification of immunoglobulins

Precipitation rings were formed around the wells charged with test samples and immunoglobulins of known concentrations. Depending upon the quantity of immunoglobulins present in the antigen well, different ring diameters were obtained (Plate 19).

Plate 19. Mancini's single radial immunodiffusion test showing different zones of precipitation

The measurements of diameter of precipitation ring formed around the antigen wells charged with known concentrations of IgG and IgM showed direct relation with the concentration of the antigens. These values were used for constructing standard curve and it gave a straight line relationship. Using this standard curve, the levels of IgM and IgG in sera samples, bile and egg yolk were assessed. The values are given in tables 16-21.

Concentration of IgM in serum of non-bursectomised and bursectomised ducklings

The concentration of IgM in pooled serum samples from non-bursectomised (control) and bursectomised ducklings of one to ten weeks of age were quantitated by Mancini's single radial immunodiffusion test. The values obtained are presented in table 16 and graphically represented in fig. 15.

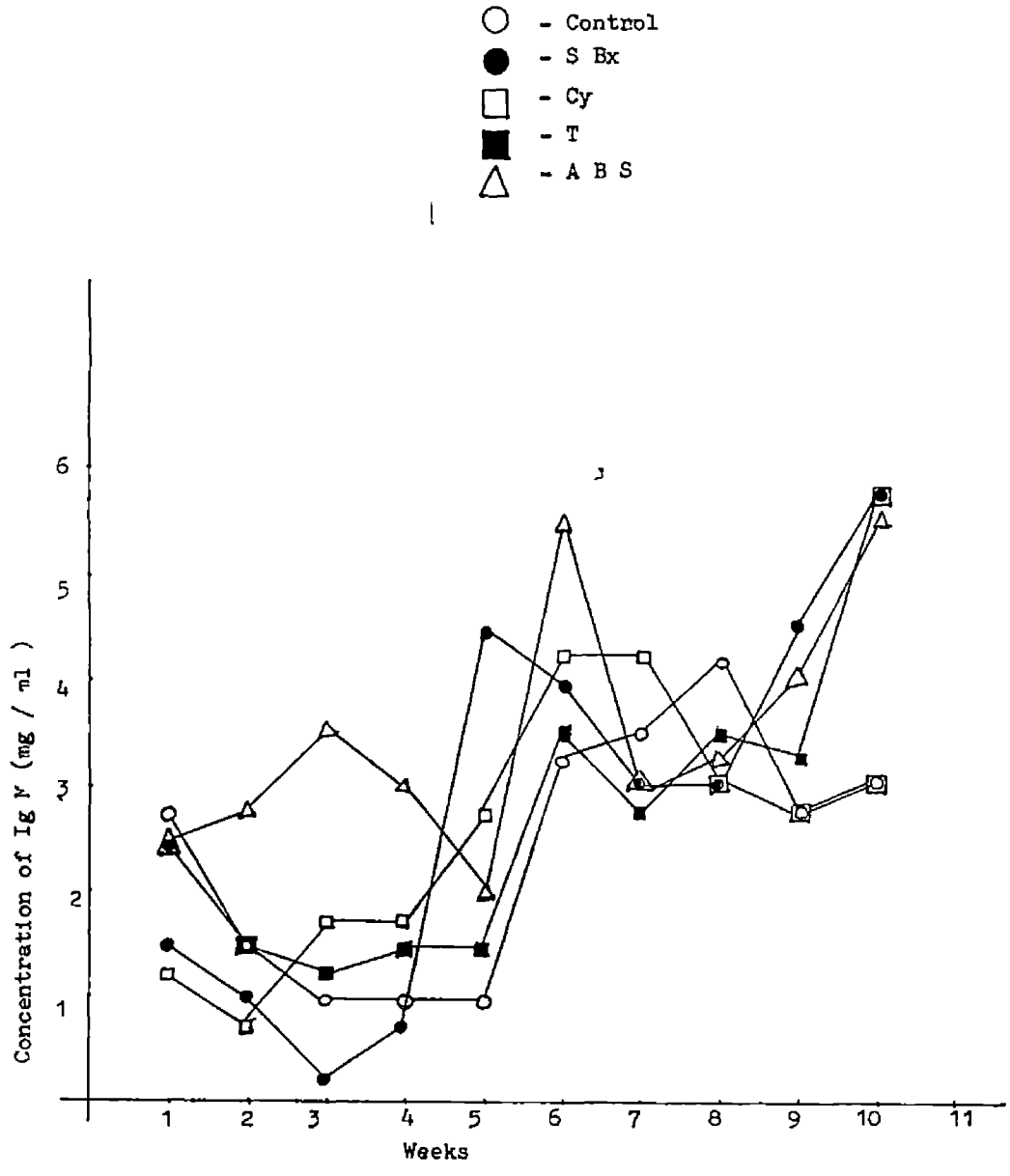
From the table values, it was found that in the non-bursectomised ducklings, the highest concentration of IgM (4.275 mg/ml) was at the eighth week of age and the lowest level (1.0 mg/ml) was at the third, fourth and fifth weeks. The IgM levels in all other weeks fell within these ranges.

In surgically bursectomised (SBx) group of ducklings, the highest concentration of serum IgM was found to be 5.0 mg/ml, in the 10th week, and the lowest level was 0.25 mg/ml in third week. The levels of IgM in first, second, third, fourth, seventh and eighth weeks were less than that of the control (Table 16).

Table 16. Serum concentration of IgM in non-bursectomised (control) and bursectomised ducklings from 1 to 10 weeks

Treatment	Concentration of IgM (mg/ml) at weeks									
	1	2	3	4	5	6	7	8	9	10
Control	2.750	1.500	1.000	1.000	1.000	3.275	3.525	4.775	2.750	3.025
SBx	1.900	1.000	0.250	0.725	4.550	4.025	3.025	3.025	4.550	5.800
Cy	1.225	0.725	1.750	1.750	2.750	4.275	4.275	3.025	2.750	3.025
T	2.300	1.200	1.225	1.500	1.500	3.525	2.750	3.525	3.275	5.900
ABS	2.500	2.750	3.525	3.025	2.000	5.550	3.025	3.275	4.025	5.550

FIG 15 MEAN SERUM IgM CONCENTRATION IN NON-BURSECTOMISED & BURSECTOMISED DUCKLINGS AGED 1 - 10 WEEKS



Cyclophosphamide treated group of ducklings showed maximum IgM level of 4.275 mg/ml in sixth and seventh weeks, which was higher than the IgM level of control at the same age. Lowest IgM level was 0.725 mg/ml, observed at second week, and was less than the level of age-matched control (1.50 mg/ml).

A maximum IgM concentration of 5.8 mg/ml and a minimum of 1.225 mg/ml were noticed in 10th and third weeks of age respectively, in testosterone treated ducklings. These values were higher than that of the age matched controls in which the values were 3.025 mg/ml and 1.0 mg/ml respectively

In the antibursal serum treated group, maximum IgM concentration (5.55 mg/ml) was observed in sixth and tenth weeks of age, which were higher than that of the controls of the same age (3.275 mg/ml and 3.025 mg/ml). The minimum concentration of IgM was found to be 2.0 mg/ml, at the fifth week, which was also higher than that of the age-matched control (1.0 mg/ml).

Among the bursectomised group, the highest level of serum IgM (5.8 mg/ml) was observed in SBx and testosterone treated ducklings, at the 10th week of age (Fig. 15). Maximum IgM level was observed at the eighth week in control (4.275 mg/ml) while in Cy treated ducklings it was seen at sixth and seventh weeks (4.275 mg/ml) and in ABS treated group at the sixth and tenth weeks (5.55 mg/ml). As it is

presented in table 16, serum IgM levels of the control and all the bursectomised groups except ABS group, were high in the first week and thereafter declined upto the third week. In the case of ABS treated ducklings, there was a gradual rise in IgM concentration from the first (2.5 mg/ml) to the third (3.525 mg/ml) week.

Serum concentration of IgM in non-bursectomised and bursectomised ducklings inoculated with SRBC/S.typhimurium

The mean serum concentration of IgM in non-bursectomised and bursectomised ducklings after inoculation with SRBC/ S. typhimurium are presented in table 17 and represented by histogram (Fig. 16-19). As in the case of total serum protein determination, here also there were three groups.

Seventh day post-inoculation

In CSR, the highest value of IgM was found in group III (3.525 mg/ml), followed by group II (3.025 mg/ml) and group I (2.75 mg/ml). In CSt also highest concentration of IgM was in group III (4.275 mg/ml), while groups I and II showed identical levels of IgM (2.5 mg/ml) (Table 17).

Among the bursectomised ducklings, group II of CySR recorded maximum IgM level (5.05 mg/ml) while group III of SBxSt recorded the minimum level (1.5 mg/ml).

Sheep RBC inoculated bursectomised ducklings of group I revealed same IgM level (3.025 mg/ml) in SBxSR, TSR and ABSR and this level was greater than that of CSR group I. In

Table 17. Serum concentration of IgM (mg/ml) in non-bursectomised and bursectomised ducklings of 3 age groups inoculated with SRBC/*S. typhimurium*

Treatment	Group I				Group II				Group III			
Days after inoculation	7	14	21	28	7	14	21	28	7	14	21	28
CSR	2.750	5.050	5.050	4.800	3.025	4.275	3.775	5.550	3.525	4.275	5.050	3.525
CSt	2.500	2.750	4.025	4.550	2.550	4.275	4.550	5.050	4.275	4.025	4.025	5.300
SBxSR	3.025	3.025	3.025	4.275	3.025	3.525	4.800	3.025	2.500	2.500	3.025	3.525
SBxSt	2.750	4.025	4.025	5.550	3.775	4.550	4.550	4.550	1.500	3.025	2.750	2.750
CySR	2.500	2.500	3.525	4.550	5.050	5.800	5.800	6.075	4.025	4.025	4.025	3.025
CySt	2.750	3.525	3.525	4.275	4.000	4.800	4.550	4.025	2.000	5.550	3.525	3.525
TSR	3.025	4.025	4.550	4.550	2.500	4.025	4.025	3.525	2.750	3.025	3.025	3.525
TSt	2.75	3.025	2.000	2.000	2.500	2.500	3.775	4.025	3.525	3.025	3.025	3.025
ABSR	3.025	3.025	3.025	4.025	3.525	4.025	5.050	5.050	4.550	2.500	2.000	2.250
ABSt	2.000	2.000	2.500	3.525	3.525	5.300	4.025	3.775	3.025	4.275	4.550	5.050

Group I - 7 day old ducklings
Group II - 28 day old ducklings
GroupIII - 42 day old ducklings

FIG 16 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgM IN NON BURSECTOMISED AND BURSE CTOMISED DUCKLINGS , 7 DAYS AFTER INOCULATION WITH SRBC / S typhimurium

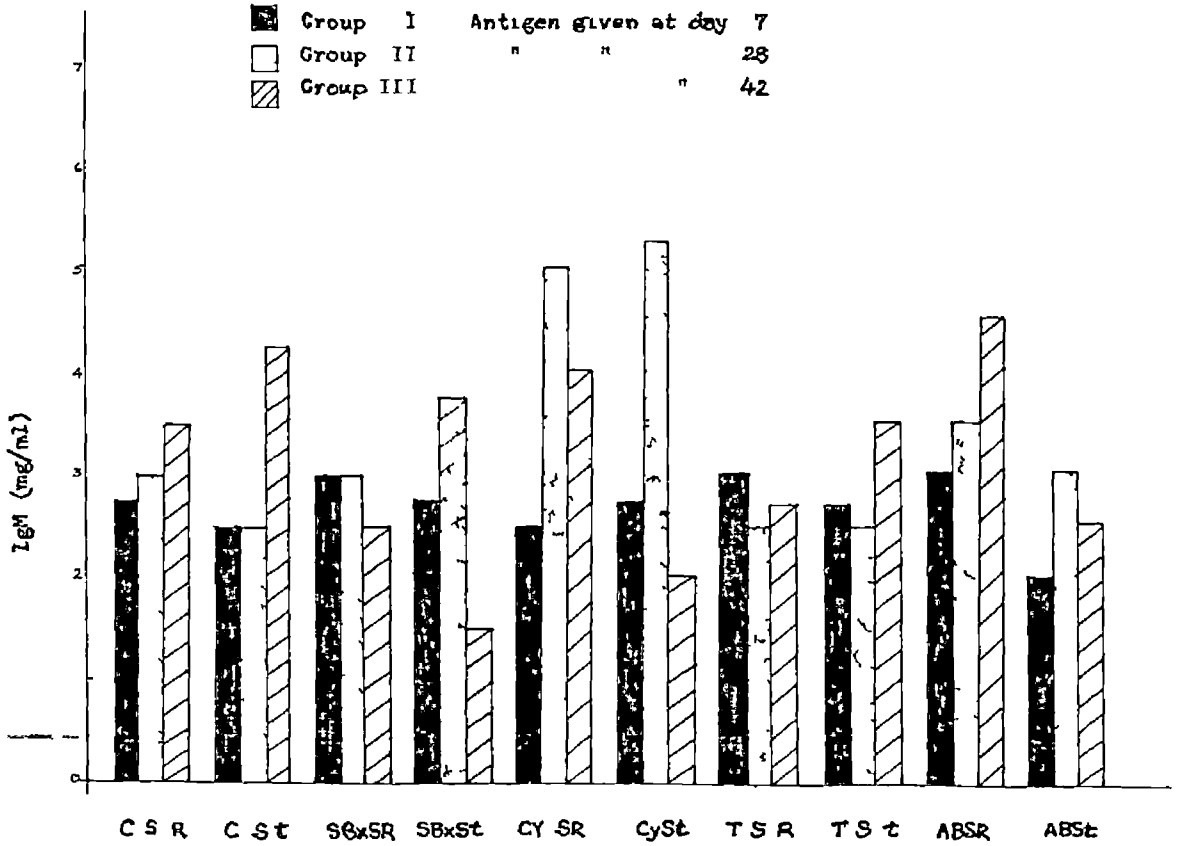


FIG 17 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgM IN NON BURSECTOMISED AND BURSECTOMISED DUCKLINGS, 14 DAYS AFTER INOCULATION WITH S R B C / S typhimurium

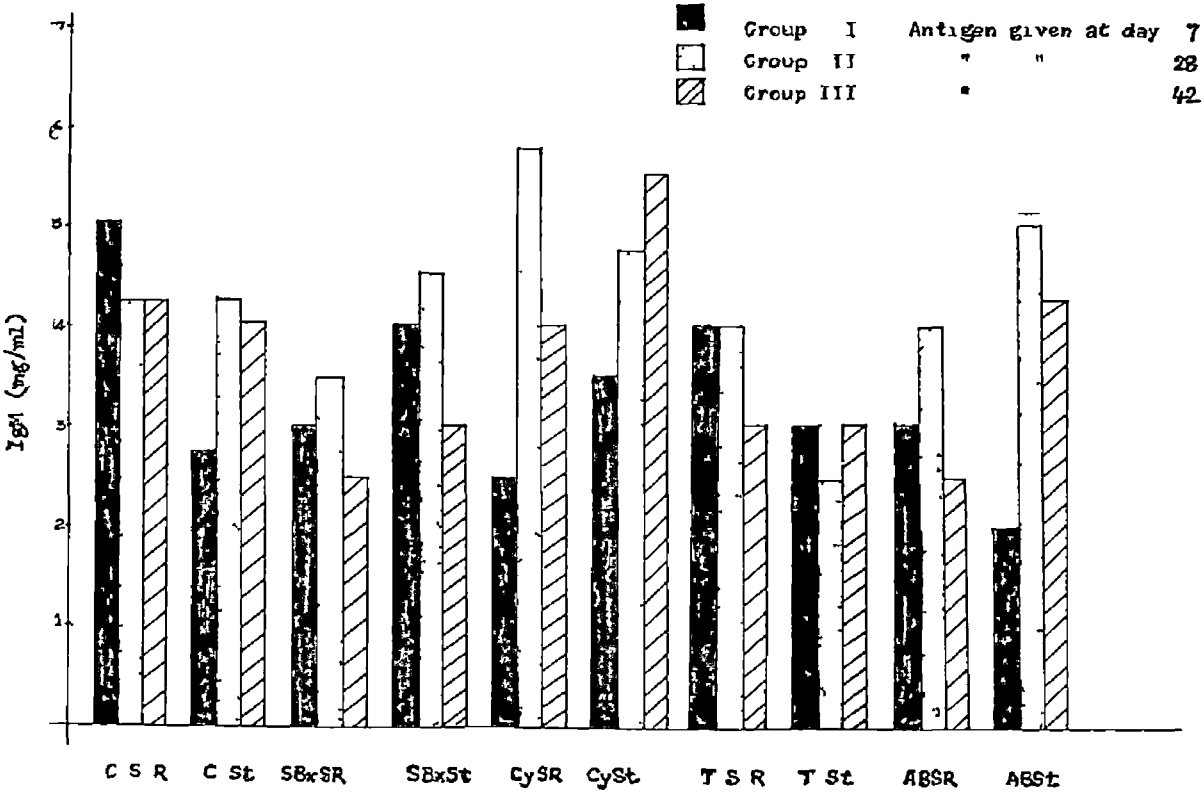


FIG 1 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgM IN NON BURSECTOMISED AND BURSECTOMISED DUCKLINGS 21 DAYS AFTER INOCULATION WITH SRBC/ S typhimurium

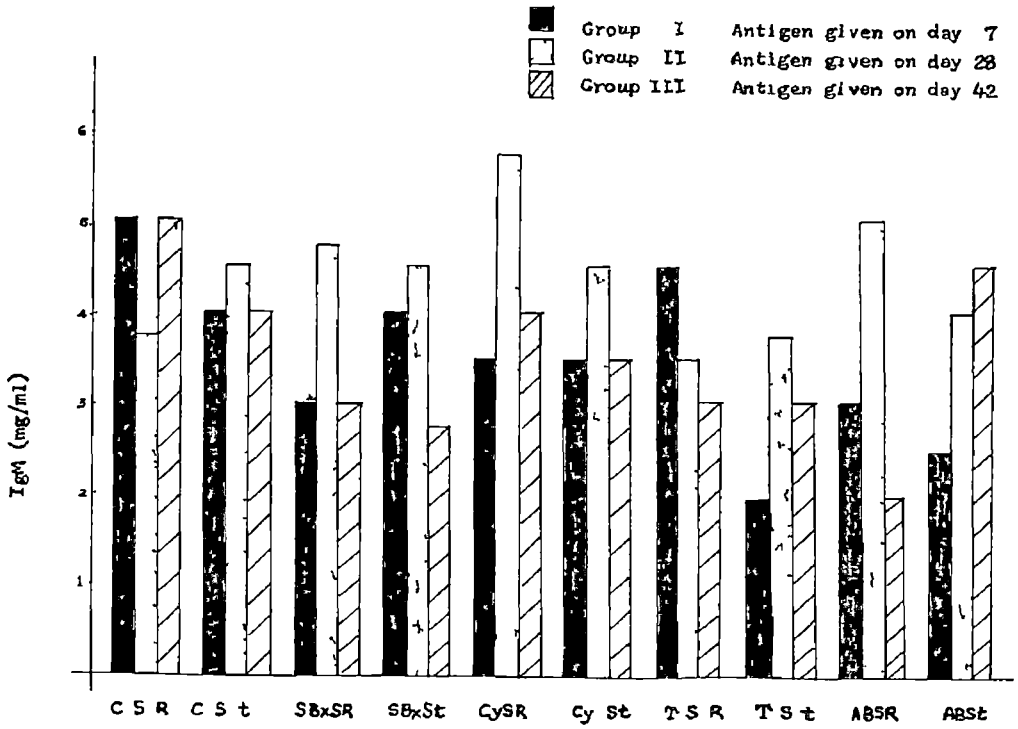
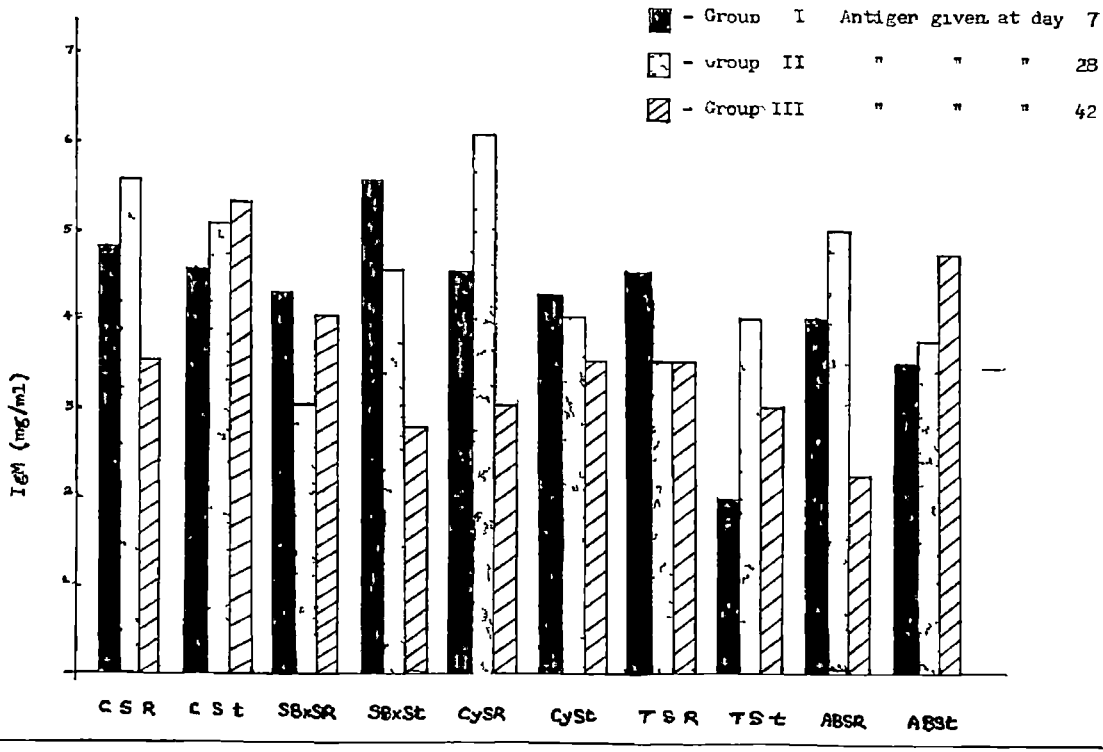


FIG 19 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgM IN NON-BURSECTOMISED AND BURSECTOMISED DUCKLINGS, 28 DAYS AFTER INOCULATION WITH S R 8 c / S typhimurium



CySR the IgM level was lower (2.5 mg/ml) than both other treatment and control groups. In group II the IgM concentration ranged from 5.05 mg/ml (CySR) to 2.5 mg/ml (TSR). Compared to CSR group II, the levels of all treated ducklings except TSR were higher. The IgM levels of group III bursectomised ducklings ranged from 4.55 mg/ml (in ABSR) to 2.5 mg/ml (in SBxSR). Compared to CSR group III, ABSR and CySR showed higher IgM values. Comparing the three groups of bursectomised birds inoculated with SR, highest IgM value was shown by group II in CySR, group III in ABSR and group I in TSR. In SBxSR groups I and II showed identical high values compared to group III (Fig. 16).

In bursectomised ducklings given S. typhimurium, in group I, IgM concentration ranged from 2.75 mg/ml (in SBxSt, CySt and TSt) to 2.0 mg/ml (in ABST). Compared to CSt group I, only the value of ABSt was lower. In group II the highest value of 4.8 mg/ml was recorded in CySt and the lowest value of 2.5 mg/ml was recorded in TSt. When compared to control (CSt group II), only TSt showed identical value while all others showed higher values. The highest IgM value in group III was 3.525 mg/ml (in TSt) and the lowest was 1.5 mg/ml (SBxSt). In all cases the IgM values of group III were lower than that of CSt group III.

On comparing the three groups of bursectomised ducklings inoculated with S. typhimurium, group II revealed

high IgM levels in CySt, SBxSt and ASSt, while group III showed highest level of IgM in TSt compared to groups I and II (Fig. 16).

Fourteenth day post-inoculation

Among non-bursectomised, SRBC inoculated ducklings, on day 14 post-inoculation, group I recorded an IgM concentration of 5.05 mg/ml, while groups II and III recorded identical values of 4.275 mg/ml. In CSt, the highest value for IgM was shown by group II (4.275 mg/ml), followed by group III (4.025 mg/ml) and group I (2.75 mg/ml) (Table 17).

In bursectomised ducklings given SRBC/*S. typhimurium*, the highest IgM level was seen in group II of CySR (5.8 mg/ml) and the lowest in group I ASSt (2.0 mg/ml).

Bursectomised ducklings of group I, given SRBC, revealed an IgM concentration ranging from 4.025 mg/ml (in TSR) to 2.5 mg/ml (in CySR). The IgM levels of all treatments under group I were lower, compared to group I of CSR. In group II, the highest value of IgM was shown by CySR (5.8 mg/ml), and the lowest value by SBxSR (3.525 mg/ml). Compared to control (CSR group II) only CySR had higher IgM value. The IgM levels in all treatments under group III were lesser than that of CSR group III and it ranged from 4.025 mg/ml (in CySR) to 2.5 mg/ml (in SBxSR and ASSR).

On comparing the three groups of bursectomised SRBC given ducklings, group II had higher IgM levels in CySR,

SBxSR and ABSR, while in TSR the IgM levels of groups I and II were identical and higher than that of group III.

Among S. typhimurium inoculated and bursectomised ducklings of group I, the IgM concentration ranged from 4.025 mg/ml (in SBxSt) to 2.0 mg/ml (in ABSt). Compared to CSt group I, the IgM levels were higher in all cases except ABSt. In group II, IgM level was maximum in ABSt (5.3 mg/ml) and minimum in TSt (2.5 mg/ml). The IgM levels were higher than that of CSt group II in all cases except TSt. IgM concentration in group III bursectomised and S. typhimurium inoculated ducklings ranged from 5.55 mg/ml (in CySt) to 3.025 mg/ml (in SSxSt and TSt). In CySt and ABSt the level was greater than that of CSt group III, while in TSt and SBxSt it was lower (Fig. 17).

Comparing the IgM levels between the three groups, group III showed the highest IgM level in CySt, while group II was having maximum IgM concentration in SBxSt and ABSt. In TSt, both the groups I and III had identical levels of IgM which was greater than that of group II (Fig. 17).

Twentyfirst day post-inoculation

On twentyfirst day post-inoculation, CSR groups I and III had identical levels of IgM (5.05 mg/ml), while group II had a lower level of 3.775 mg/ml. In CSt, highest IgM level was shown by group II (4.55 mg/ml), while groups I and III showed identical levels of 4.025 mg/ml each (Table 17).

In bursectomised ducklings inoculated SRBC/S.typhimurium maximum IgM concentration was shown by group II of CySR (5.8 mg/ml) and the minimum by group I of TSt and group III of ABSR (2.0 mg/ml).

Among the bursectomised ducklings administered SRBC, the group I had highest IgM level in TSR (4.53 mg/ml) and the lowest in SBxSR and ABSR (3.025 mg/ml). Compared to CSR group I the IgM values of all treated group I birds were lower. In group II, the IgM ranged from 5.8 mg/ml (in CySR) to 4.025 mg/ml (in TSR). In all cases, IgM values were higher than that of CSR group II (Fig. 18). IgM concentration in group III ranged from 4.025 mg/ml (in CySR) to 2.0 mg/ml ((in ABSR). Compared to CSR group III, the IgM levels of treated group III birds were lower.

On comparison of the IgM levels between the three groups of SRBC treated birds, group II showed highest concentration of IgM in SBxSR, CySR and ABSR, while in TSR, group I showed highest concentration.

In S. typhimurium inoculated bursectomised ducklings, group I showed highest IgM level (4.025 mg/ml) in CBxCt and TSt showed the lowest level (2.0 mg/ml). Compared to CSt group I, the IgM level of SBxSt was the same, while that of the CySt, TSt and ABSt were lower (Fig. 18). In group II, highest level of IgM was in CBxCt and CySt (4.55 mg/ml) and the lowest level was seen in TSt (3.775 mg/ml). Compared to

control (CSt group II), the IgM levels of CySt and SBxSt were identical, while that of TSt and ABSt were lower. The IgM concentration in group III ranged from 4.55 mg/ml (in ABSt) to 2.75 mg/ml (in SBxSt). Only ABSt group III showed higher IgM level than CSt group III, while in others it was lower.

On comparison within the groups, group II showed highest IgM values in SBxSt, CySt and TSt, while group III showed highest value in ABSt.

Twentyeighth day post-inoculation

On the 28th day post-inoculation, CSR group II showed the highest IgM value (5.55 mg/ml), followed by group I (4.8 mg/ml) and group III (3.525 mg/ml). In CSt the highest IgM concentration was shown in group III (5.3 mg/ml) and the lowest in group I (4.55 mg/ml). Group II showed an intermediary value of 5.05 mg/ml (Table 17).

In the bursectomised and SRBC/S. typhimurium inoculated ducklings, group II CySR recorded the maximum IgM level (6.075 mg/ml) and group I TSt recorded the minimum level (2.0 mg/ml).

Group I of bursectomised and SRBC administered ducklings revealed the highest IgM value in CySR and TSR (4.55 mg/ml) and the lowest value in ABSR (4.025 mg/ml). On comparison with CSR group I, the IgM levels in all four treatments were lower. In group II, the maximum IgM concentration was shown

by CySR (6.075 mg/ml) and the lowest level by SBxSR (3.025 mg/ml) when compared to CSR group II, only, CySR showed a higher IgM value (Fig. 19). The IgM value ranged 3.525 mg/ml (in SBxSR and TSR) to 2.25 mg/ml (in ABSR), in group III of bursectomised and SRBC given ducklings. The IgM values of SBxSR, TSR and CSR were identical in group III (3.525 mg/ml), while it was lower in CySR (3.025 mg/ml) and ABSR (2.25 mg/ml).

On comparing groups I to III, group I had the highest IgM levels in SBxSR and TSR, while group II showed highest levels in CySR and ABSR.

Among S. typhimurium administered bursectomised ducklings of group I, SBxSt revealed highest IgM value (5.55 mg/ml), while TSt had the lowest value (2.0 mg/ml). The IgM level of only SBxSt was higher than that of CSt group I. In group II, IgM values ranged from 4.55 mg/ml (in SBxSt) to 3.775 mg/ml (in ABSt). The IgM levels in all treatments were lower than that of CSt group II. On comparing group III treatments the highest IgM level was obtained for ABSt (5.05 mg/ml) and the lowest for SBxSt (2.75 mg/ml). All the values were lower, compared to CSt group III (Fig. 19).

A comparison between groups revealed highest levels of IgM in group I of SBxSt and CySt, while in TSt it was group II and in ABSt, group III, which showed the highest levels of IgM.

Serum concentration of IgG in non-bursectomised and bursectomised ducklings

Pooled serum samples from non-bursectomised (control) and bursectomised ducklings of one to ten weeks of age were quantitated for IgG level by Mancini's single radial immunodiffusion test. The results obtained are presented in table 18 and graphically represented in Fig. 20.

Table value revealed that in control ducklings, the highest concentration of serum IgG (8.975 mg/ml) was observed in eighth and tenth weeks of age, and the lowest level (5.975 mg/ml) was observed at ninth week of age. The level of serum IgG at the first week was 7.475 mg/ml (Table 18).

In surgically bursectomised group of ducklings, the highest serum IgG level (8.475 mg/ml) was seen at fifth, ninth and tenth weeks of age. This value was less than the highest level of IgG seen in control. The lowest IgG value in this group was 4.0 mg/ml at the fourth week, which was also lesser than the value for control (5.975 mg/ml).

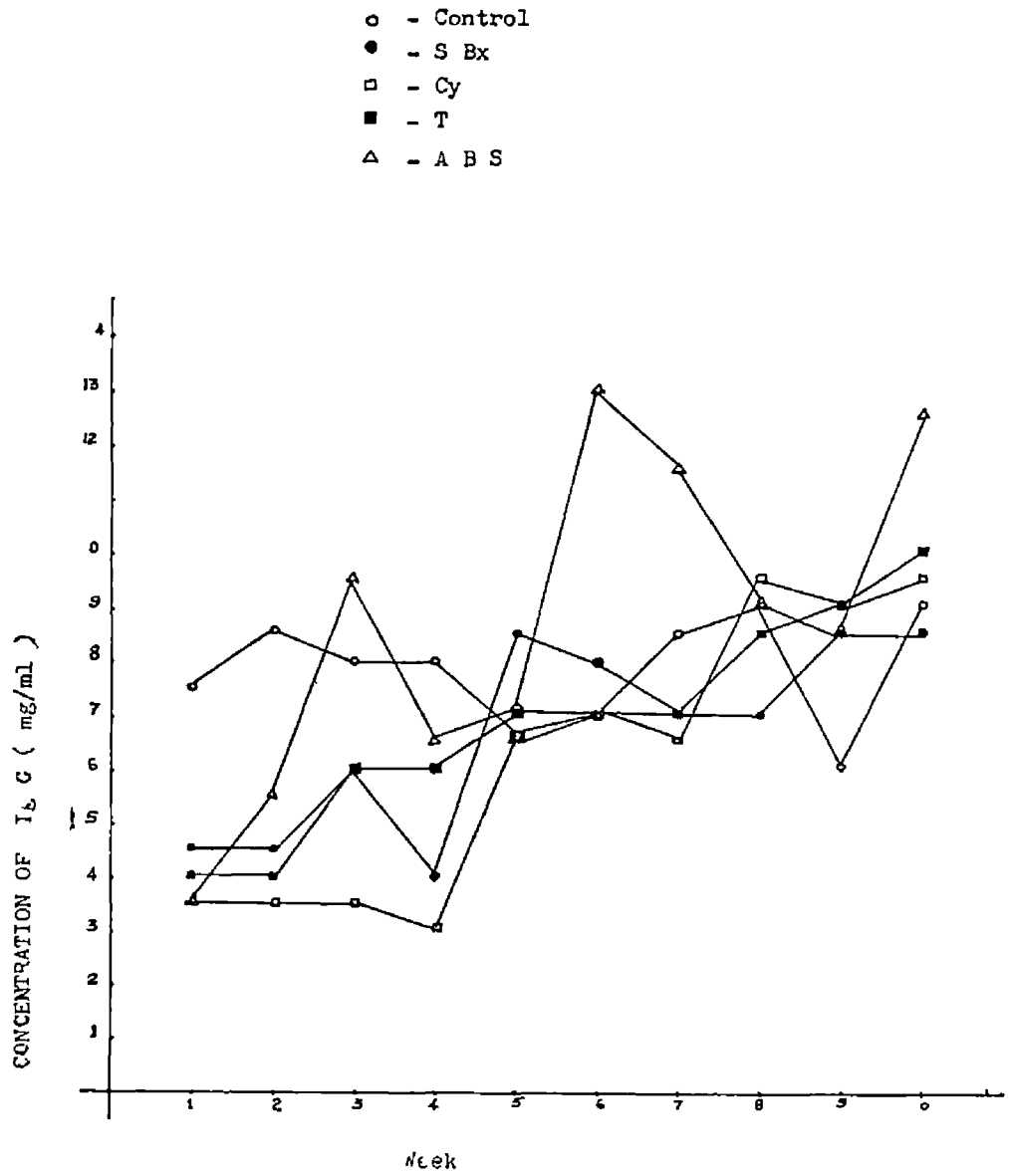
Cyclophosphamide treated ducklings revealed a maximum IgG level (9.5 mg/ml) at the eighth and tenth weeks of age, which was greater than the maximum value for control. The minimum IgG level was 3.0 mg/ml at the fourth week, which was less than that of the control.

In testosterone treated ducklings, tenth week showed the maximum level (10.0 mg/ml) of IgG, while the minimum value

Table 18. Serum concentration of IgG in non-bursectomised and bursectomised ducklings from 1 to 10 weeks

Treatments	Concentration of IgG (mg/ml) at weeks									
	1	2	3	4	5	6	7	8	9	10
Control	7.475	8.475	8.000	8.000	6.500	7.000	8.475	9.975	5.975	8.975
Bbx	4.475	4.475	5.475	4.000	8.475	8.000	7.000	7.000	6.475	8.475
Cy	3.475	3.475	3.475	3.000	6.500	7.000	6.500	9.500	8.975	9.500
T	4.000	4.000	5.475	5.475	7.000	7.000	7.000	8.475	8.975	10.000
BS	3.475	5.475	8.300	6.500	7.000	13.050	11.525	8.975	8.475	12.550

FIG 20 MEAN SERUM IgG CONCENTRATION IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS AGED 1 10 WEEKS



(4.0 mg/ml) was seen in first and second weeks. Compared to control, as in Cy treated group, the highest IgG level was greater and the lowest level was lesser, in testosterone treated group also.

Antibursal serum administered group of ducklings revealed the highest IgG level (13.05 mg/ml), when compared to control and other treated groups. The lowest value in this case was 3.475 mg/ml, seen in first week, which was less than the control minimum.

Among the bursectomised groups, maximum concentration of serum IgG was observed in ABS given group (13.05 mg/ml) at sixth week and the minimum (3.0 mg/ml) in Cy treated group at the fourth week. In SBx, Cy and testosterone treated groups, maximum serum IgG levels were showed at the tenth week (Fig. 20). In the first week, IgG level was highest in control(7.475 mg/ml), when compared to SBx (4.475 mg/ml), Cy (3.475 mg/ml), testosterone (4.0 mg/ml) or ABS (3.475 mg/ml) groups.

Serum concentration of IgG in non-bursectomised and bursectomised ducklings inoculated with SRBC/S.typhimurium

As in the case of mean concentration of IgM, the concentration of IgG in non-bursectomised and bursectomised ducklings was determined after inoculation with SRBC/ S. typhimurium, by Mancini's single radial immunodiffusion technique. The values of serum IgG obtained are presented in table 19 and represented by histograms in Figs. 21-24.

Table 19. Serum concentration of IgG (mg/ml) in non-bursectomised and bursectomised ducklings of 3 age groups inoculated with SRBC/*S.typhimurium*

Treatment	Group I				Group II				Group III			
Days after inoculation	7	14	21	28	7	14	21	28	7	14	21	28
CSR	4.475	5.000	5.000	8.375	5.000	6.500	7.475	8.975	3.000	7.000	8.000	8.975
CSt	3.000	3.000	4.000	8.475	4.000	5.475	8.000	8.475	5.475	8.975	8.475	11.025
SBxSR	3.475	5.000	5.000	5.175	5.975	6.500	8.000	8.975	7.000	7.475	7.475	8.000
SBxSt	4.000	5.000	11.025	11.025	5.975	9.475	10.000	5.975	8.000	7.000.	7.000	6.500
CySR	3.175	3.475	5.000	4.475	8.000	8.975	8.000	8.000	5.975	6.500	5.975	5.975
CySt	3.475	5.000	5.000	5.000	5.975	7.475	8.475	8.000	7.000	8.975	7.475	7.475
TSR	3.000	3.000	5.000	5.475	4.175	7.000	9.500	10.000	7.475	8.975	9.500	10.000
TSt	3.475	5.000	5.000	5.000	5.475	5.975	8.975	10.000	8.975	10.525	10.525	11.025
ABSR	3.000	3.000	4.000	7.000	7.000	5.975	7.475	10.525	8.000	3.000	3.000	3.000
ABSt	3.175	3.475	4.000	5.975	5.000	7.000	7.475	9.500	5.975	6.500	7.475	12.050

Group I - 7 day-old ducklings
Group II - 28 day-old ducklings
Group III - 42 day-old ducklings

FIG 21 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgG IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS , 7DAYS AFTER INOCULATION WITH SRBC / S typhimurium

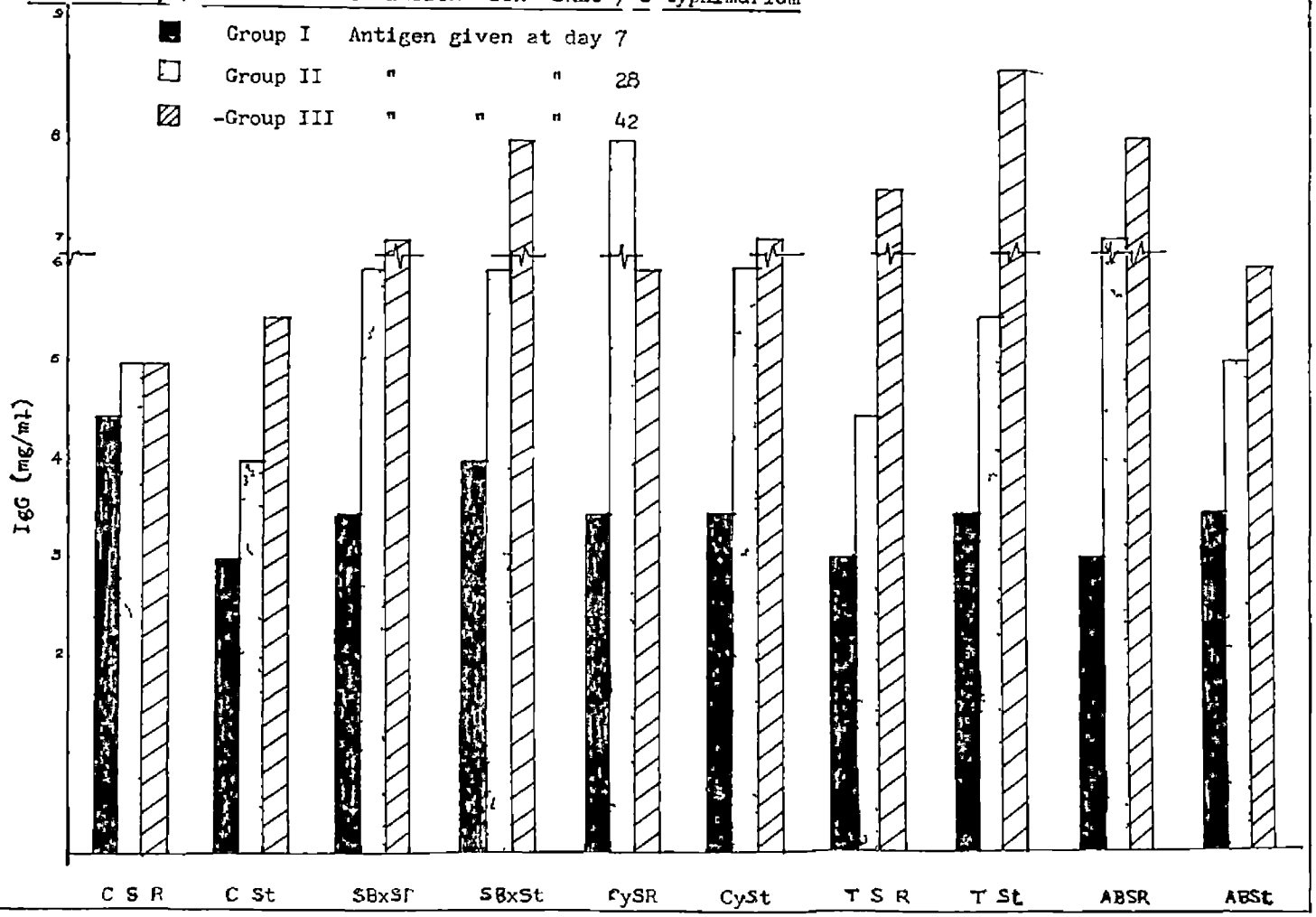


FIG.22 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgG IN NON-BURSECTOMISED AND BURSECTOMISED DUCKLINGS , 14 DAYS AFTER INOCULATION WITH SRBC/ S typhimurium

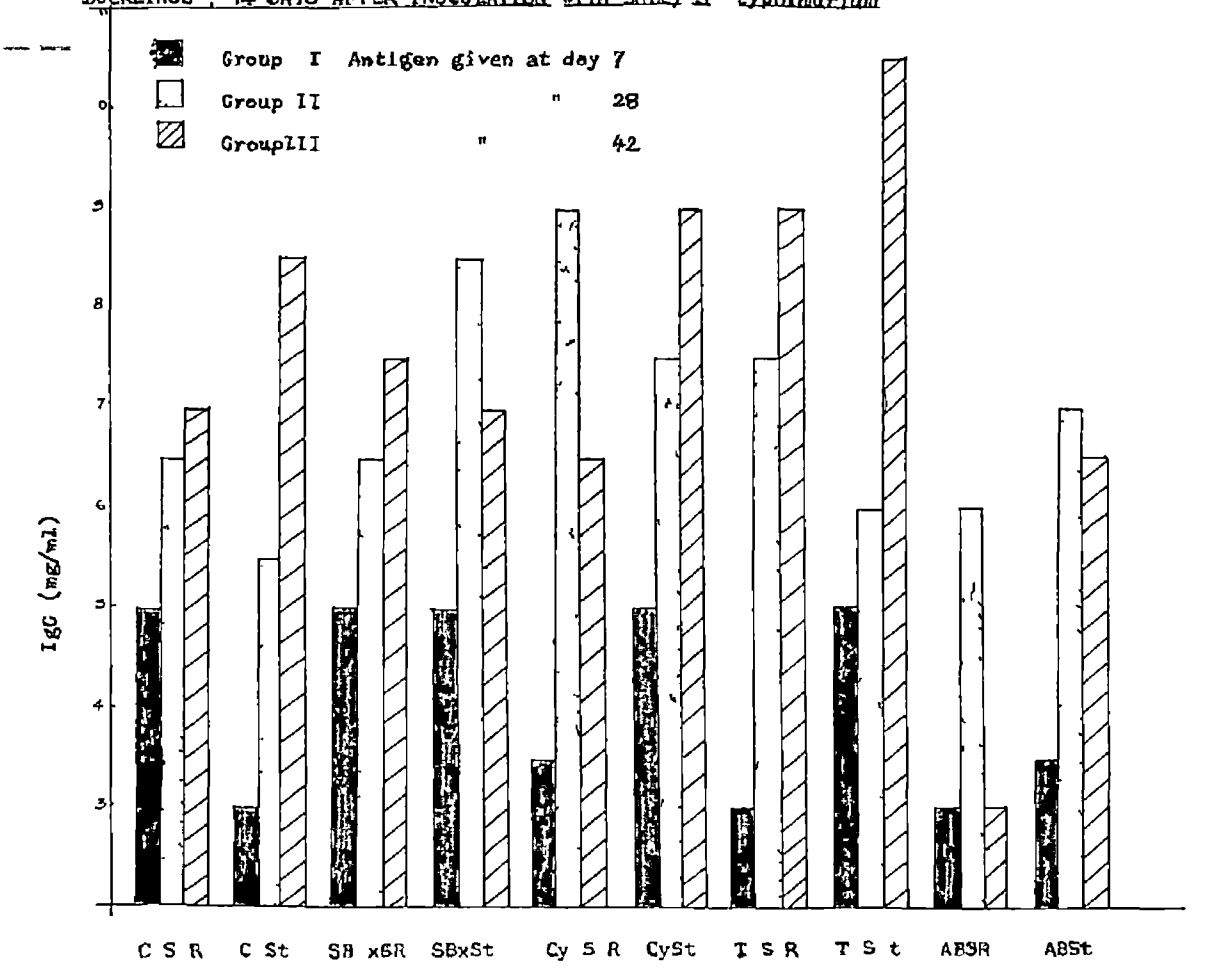


FIG 23 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgG IN NON BURSECTOMISED AND BURSECTOMISED DUCKLINGS, 21 DAYS AFTER INOCULATION WITH S R B C /S typhimurium

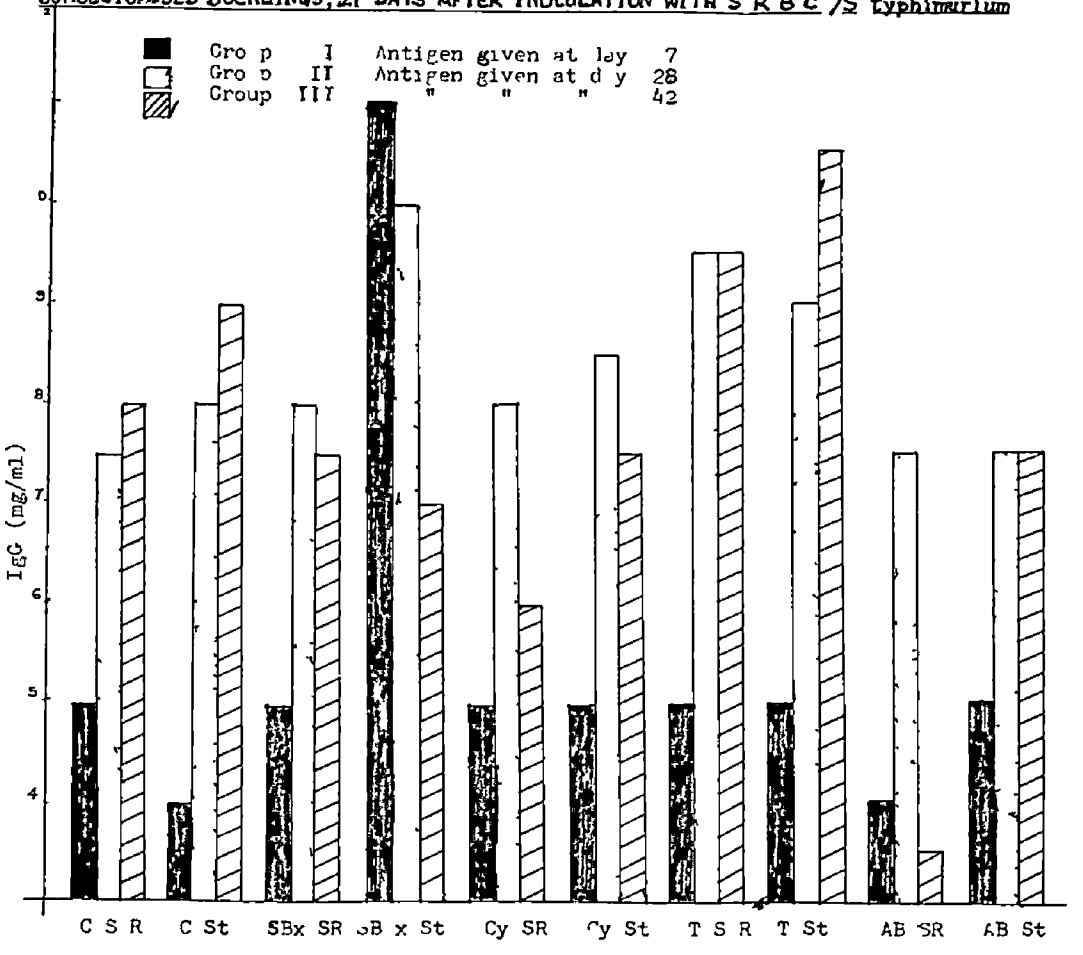
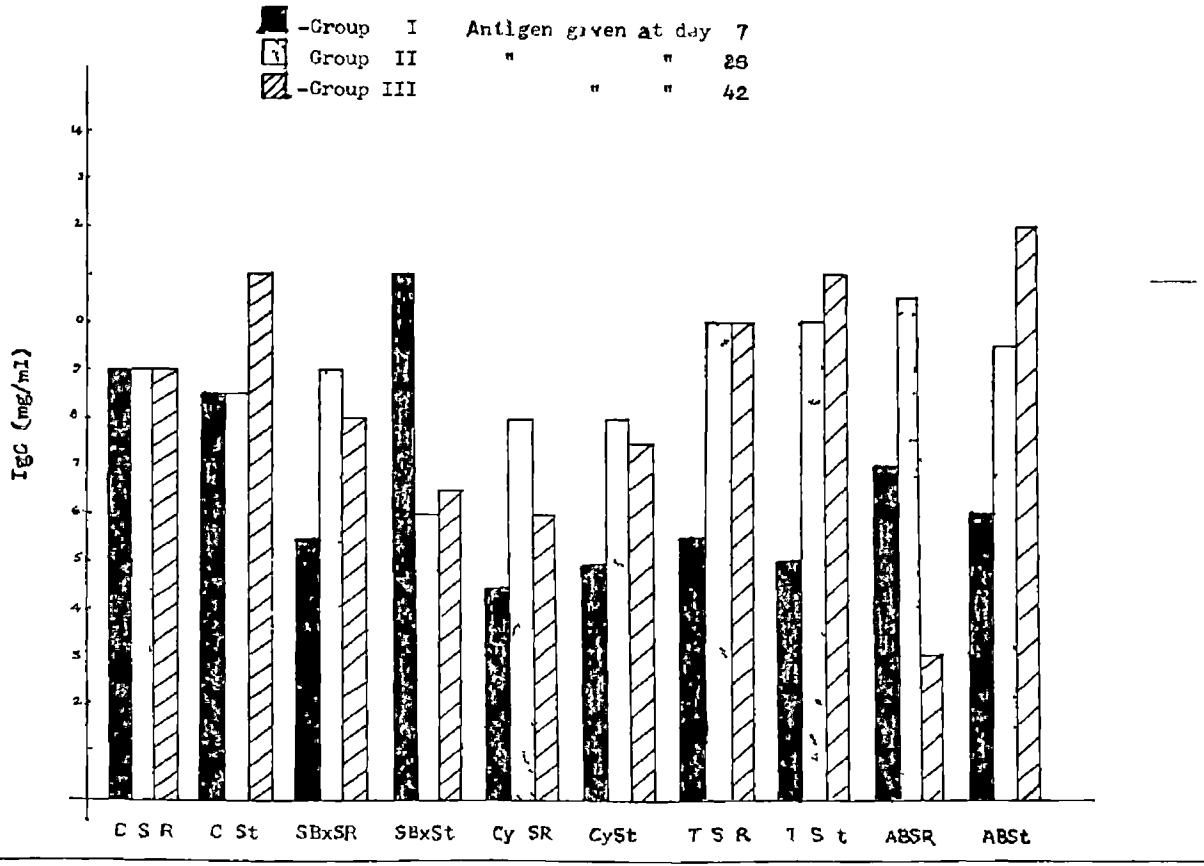


FIG 24 HISTOGRAM REPRESENTING MEAN SERUM CONCENTRATION OF IgG IN NON BURSECTOMISED & BURSECTOMISED DUCKLINGS 28 DAYS AFTER INOCULATION WITH S R B C /S typhimurium



Seventh day post-inoculation

On day seven post-inoculation the maximum IgG level (5.0 mg/ml) was shown by groups II and III of CSR, while group I showed a value of 4.475 mg/ml. In CSt, the highest IgM level was seen in group III (5.475 mg/ml), followed by group II (4.0 mg/ml) and group I (3.0 mg/ml) (Table 19).

Among the bursectomised ducklings inoculated with SRBC/ S. typhimurium, the maximum IgG level was observed in group III of TSt (8.975 mg/ml) and the minimum value in group I of TSR and ABSR (3.0 mg/ml).

In bursectomised and SRBC given ducklings of group I, the highest level of IgG was seen in SBxSR and CySR (3.475 mg/ml) and lowest level was seen in TSR and ABSR (3.0 mg/ml). All these levels were lower than that of CSR group I. In group II, IgG level ranged from 8.0 mg/ml (in CySR) to 4.475 mg/ml (in TSR). The IgG levels of both SBxSR and CySR were higher than that of group II CSR, while the levels of TSR and ABSR were lower. IgG concentration in group III ranged from 8.0 mg/ml (in ABSR) to 5.975 mg/ml (in CySR). Compared to CSR group III, all treated ducklings showed higher IgG levels.

On comparing within the three groups of bursectomised and SRBC given ducklings, group III was found to have the maximum IgG level in SBxSR, TSR and ABSR, while in CySR, group II showed maximum level (Fig. 21).

Group I bursectomised ducklings inoculated with S. typhimurium recorded highest level of IgG in SBxSt (4.0 mg/ml), while identical levels of 3.475 mg/ml were detected in other three treatments. The levels of IgG were higher in all cases than that of CSt group I. In group II, IgG values ranged from 5.975 mg/ml (in SBxSt and CySt) to 5.0 mg/ml (in ABSt). In this case also, the IgG level was higher in all treatments, compared to CSt group II. Concentration of IgG in group III ranged from 0.975 mg/ml (in TSt) to 5.975 mg/ml (in ABSt). As in the above cases, in group III also the IgG values were higher in the treated groups, when compared to CSt group III.

On comparing the three groups in bursectomised and S. typhimurium inoculated ducklings, group III was found to give maximum IgG levels in all four treatments.

Fourteenth day post-inoculation

At the 14th day post-inoculation, in CSt, maximum IgG concentration was seen in group III (7.0 mg/ml), followed by group II (6.5 mg/ml) and group I (5.0 mg/ml). In CSt ducklings also maximum IgG concentration was recorded in group III (6.475 mg/ml), followed by group II (5.475 mg/ml) and group I (3.0 mg/ml) (Table 19).

Among the bursectomised ducklings inoculated with S-BDC/ S. typhimurium, TSt group III showed the highest level of serum IgG (10.525 mg/ml) while the lowest IgG level of

3.0 mg/ml was seen in group I of TSR and groups I and III of ABSR.

In bursectomised ducklings given SRBC, the maximum concentration of IgG in group I was seen in SBxSR (5.0 mg/ml) and the minimum in TSR and ABSR (3.0 mg/ml). Only SBxSR showed the same level of IgG compared with CSR group I, while in others, the levels were lower. In group II, IgG value ranged from 8.975 mg/ml in CySR to 5.975 mg/ml in ABSR. Compared to CSR group II, CySR and TSR revealed higher IgG values, while SBxSR showed similar, and ABSR, lower values (Fig. 22). The concentration of IgG in group III ranged from 8.975 mg/ml (in TSR) to 3.0 mg/ml (in ABSR). Only SBxSR and TSR of group III showed higher IgG concentration than CSR group III. Comparing between the three groups, in SBxSR and TSR, maximum IgG values were obtained in group III, while in CySR and ABSR, group II showed maximum value.

S. typhimurium given bursectomised ducklings of group I had a highest IgG level of 5.0 mg/ml in SBxSt, CySt and TSt, and lowest level of 3.475 mg/ml in ABSt. All these values were higher than that of CSt group I. Group II ducklings showed an IgG level ranging from 8.475 mg/ml (in SBxSt) to 5.975 mg/ml (in TSt). In this case also, all the values of the bursectomised birds were higher than those for CSt group II. Maximum IgG level in group III bursectomised and S. typhimurium inoculated ducklings was seen in TSt (10.525 mg/ml) and the minimum in ABSt (6.5 mg/ml). The

IgG levels of group III, when compared to CSt group III, were higher in CySt and TSt only (Fig. 22). On comparing the three groups, group II had highest IgG levels in SBxSt and ABSt, while group III IgG level was maximum in CySt and TSt.

Twentyfirst day post-inoculation

The maximum IgG level in CSR on 21st day post-inoculation was in group III (8.0 mg/ml), followed by group II (7.475 mg/ml) and group I (5.0 mg/ml). In CSt also the maximum IgG level was in group III (8.975 mg/ml), followed by group II (8.0 mg/ml) and group I (4.0 mg/ml (Table 19).

Among the bursectomised ducklings, the highest level of IgG was observed in group I of SBxSt (11.025 mg/ml) and the lowest in group III of ABSR (3.0 mg/ml).

Group I of bursectomised ducklings inoculated with SRBC showed an IgG range from 5.0 mg/ml (in SBxSR, CySR and TSR), to 4.0 mg/ml (in ABSR). The IgG levels of SBxSR, CySR and TSR were identical with that of CSR group I. In group II, highest IgG value was given by TSR (9.5 mg/ml) and lowest by ABSR.(7.475 mg/ml). The IgG levels were the same in CySR and SBxSR (8.0 mg/ml). In comparison with CSR group II, ABSR gave identical value, while the others gave higher values. The value for IgG was highest in TSR group III (9.5 mg/ml) and lowest in ABSR group III (3.0 mg/ml). Only TSR showed a higher IgG value in group III, on comparison with CSR group III.

In comparing groups I to III of bursectomised and SRBC inoculated ducklings, group II showed maximum IgG levels in all the treated groups, while in TSR, group III also showed the same value as that of group II (Fig. 23).

Bursectomised ducklings of group I, inoculated with S. typhimurium, showed highest IgG concentration in SBxSt (11.025 mg/ml) and lowest in ABSt (4.0 mg/ml). The IgG values of SBxSt, CySt and TSt were higher than that of CSt group I, while ABSt was identical with control. In group II, the IgG concentration ranged from 10.0 mg/ml in SBxSt to 7.475 mg/ml in ABSt. Only ABSt group II had lower IgG value compared to CSt group II, while SBxSt, CySt and TSt showed higher values. The concentration of IgG was highest in TSt group III (10.525 mg/ml).and lowest in SBxSt group III (7.0 mg/ml). Only TSt showed higher value than CSt group III, while SBxSt, CySt and ABSt showed lower IgG values.

On comparing the three groups of bursectomised and S. typhimurium inoculated ducklings, highest IgG value was observed in group I of SBxSt, group II of CySt and group III of TSt. In ABSt, groups II and III showed equal quantities of IgG (Fig. 23).

Twentyeighth day post-inoculation

On the 28th day post-inoculation, the mean IgG levels in CSR were the same in all three groups (8.975 mg/ml). In CSt maximum IgG level was shown by group III (11.025 mg/ml), while

groups I and II showed same IgG level (8.475 mg/ml) (Table 19).

The maximum level of IgG among bursectomised ducklings inoculated with SRBC/*S. typhimurium* was observed in group III of ABSt (12.05 mg/ml) and the minimum level in group III of ABSR (3.0 mg/ml).

In SRBC inoculated, bursectomised birds, group I showed an IgG range from 7.0 mg/ml (in ABSR) to 4.475 mg/ml (in CySR). All four treatments were having lower IgG levels, compared to CSR group I. In group II, ABSR revealed maximum IgG concentration (10.525 mg/ml), while CySR showed the minimum level (8.0 mg/ml). Compared to CSR group II, TSR and ABSR had higher IgG values, SBxSR had same IgG level and CySR had lower level (Fig. 24). Highest IgG concentration in group III was observed in TSR (10.0 mg/ml) and the lowest in ABSR (3.0 mg/ml). In comparison with CSR group III, only TSR had higher IgG level, while SBxSR, CySR and ABSR had lower levels.

On comparing groups I to III of SRBC inoculated bursectomised birds, group II showed maximum IgG levels in all four treatments, but in TSR, group III also had the same IgG level.

Group I of *S. typhimurium* inoculated and bursectomised ducklings revealed an IgG concentration ranging from 11.025 mg/ml (in SBxSt) to 5.0 mg/ml (in CySt and TSt). On comparing with CSR group, only SBxSt showed higher IgG level, while CySt, TSt and ABSt showed lower levels (Fig. 24). In group II, TSt recorded a maximum IgG level (10.0 mg/ml),

while SBxSt showed minimum level (5.975 mg/ml). Compared to CSt group II, TSt and ABSt showed higher IgG values, whereas SBxSt and CySt had lower values. Maximum IgG concentration in group III was observed in ABSt (12.05 mg/ml) and minimum in SBxSt (6.5 mg/ml). In comparison with CSt group III, only ABSt had a higher IgG level, while TSt showed comparable IgG level and SBxSt and CySt had lower levels.

Comparing the three groups in S. typhimurium inoculated-bursectomised birds, group III showed maximum IgG values in TSt and ABSt, while in SBxSt it was group I and in CySt, group II, which showed maximum values (Fig. 24).

Concentration of immunoglobulins in bile and egg yolk

The concentration of immunoglobulins in bile and egg yolk was determined by Mancini's single, radial immunodiffusion technique, using specific antisera for IgM and IgG.

On quantitating the immunoglobulins in bile of non-bursectomised (control) and bursectomised ducklings of eight weeks of age, precipitation rings were produced only against anti-IgM, while no ring formation occurred against anti-IgG. This indicated the presence of only IgM in bile of ducklings. The mean IgM concentrations in control and treated ducklings are given in table 20. From the table values it was evident that while control ducklings showed a high concentration of IgM (4.025 mg/ml), the IgM concentration was greatly reduced in SBx (2.0 mg/ml), Cy treated (1.0 mg/ml) and testosterone

Table 20. Concentration of IgM in bile of non-bursectomised and bursectomised ducklings of 8 weeks of age

Treatment	Concentration of IgM (mg/ml)
Control	4.025
SBx	?.000
Cy	1.000
T	2.750
ABS	3.025

Table 21. Concentration of IgM and IgG in egg yolk

Sample No.	IgM (mg/ml)	IgG (mg/ml)
1	3.220	8.000
2	3.220	7.100
3	4.440	7.100
4	2.820	9.640
Mean	3.425	8.000

treated (2.75 mg/ml) groups. In ABS group the IgM concentration compared to control was only 3.025 mg/ml.

Four egg yolk samples were used for determining the Ig level in egg yolk. The values obtained are presented in table 21. In this case, precipitation rings were produced against both anti-IgM and anti-IgG. From the table values the IgM level in egg yolk was found to be in the range of 2.02 mg/ml to 4.44 mg/ml, with a mean concentration of 3.425 mg/ml. The IgG concentration ranged from 7.16 mg/ml to 9.64 mg/ml, with a mean value of 8.0 mg/ml.

Total leucocyte count

The total leucocyte counts of non-bursectomised (control) and bursectomised ducklings were determined from one to ten weeks of age and the results are presented in table 22. The bursectomised ducklings comprised of surgically bursectomised (BSx), cyclophosphamide treated (Cy), testosterone treated (T) and antibursal serum administered (ABS) groups.

In the control group, the highest mean leucocyte count was observed in the first week (43,280/cmm), with a range of 37,000-49,000/cmm. The lowest mean leucocyte count was seen in the third week (30,885/cmm), with a range of 24,925-36,125/cmm. The values for the other weeks fell within these ranges.

Bursectomised ducklings revealed the highest mean leucocyte count (43,590/c.mm) which was higher than that

Table 22. Mean total leucocytic count of non-bursectomised and bursectomised ducklings at weekly intervals

Treatments	Age in weeks									
	1	2	3	4	5	6	7	8	9	10
Control	45280	44120	30685	39345	43030	43730	43430	39610	41320	35900
SBx	44700	45590	29700	32260	42250	44290	39880	40650	45338	35800
Cy	39000	41630	20380	32980	44040	43600	41000	41570	44250	44933
T	43720	43090	43040	42140	40720	43300	43950	43538	44750	40450
ABO	36550	36640	43760	42040	38080	40560	43350	39150	41950	43900

of the control (44,120/c.mm) in the second week of SBx group and the lowest mean leucocyte count (28,360/c.mm) in the third week of Cy group. The lowest value was lesser than that obtained for age matched control (30,885/c.mm) (Table 22).

In surgically bursectomised ducklings, the highest mean leucocyte count was 45,590/c.mm (range 41,500-49,250/c.mm) recorded in the second week, and the lowest count was 29,700/c.mm (range 20,900-35,400/c.mm), seen in the third week.

Cyclophosphamide treated ducklings revealed the maximum mean leucocyte count of 44,940/c.mm (range 40,000-49,500/c.mm) in the fifth week and the minimum count of 28,360/c.mm (range 21,450-39,150/c.mm) in the third week.

In testosterone treated group, highest count of leucocytes was seen in the ninth week (44,750/c.mm, with a range of 44,000-45,200/c.mm), while the lowest count was obtained in the tenth week (40,450/c.mm with a range of 35,000-45,200/c.mm).

At the tenth week of age, antibursal serum administered group recorded the maximum leucocyte count of 43,900/c.mm (range 40,000-48,000/c.mm), whereas the minimum count was recorded at the fifth week (36,080/c.mm with a range of 24,000-42,000/c.mm).

Among the five groups of ducklings, a decrease in total leucocyte count was observed in the third week, in control,

SBx and Cy groups, while in testosterone treated and ABS groups, an increased count was seen in third week, compared to the second week. In the seventh week also, control, SBx and Cy groups showed decrease in leucocyte count, compared to sixth week, while testosterone and ABS treated groups had higher counts. At the tenth week, lower leucocyte counts were obtained for control, SBx and testosterone groups, while Cy and ABS groups had higher counts, compared to ninth week (Table 22).

Statistical analysis of the data is given in table 23. A comparison of week-wise total leucocyte counts revealed significant difference ($P<0.01$) between the testosterone treated group (43,840/c.mm) and the SBx (29,700/c.mm) and Cy (28,360/c.mm) groups in the third week of age. But no significant difference was detected between testosterone and ABS treated groups, at the third week. No significant differences were observed between the groups at other weeks.

Differential count

The differential leucocyte count of the non-bursectomised (control) and bursectomised ducklings from one to ten weeks of age was determined. As in the case of total leucocyte count, four treatment groups were there in surgically bursectomised (SBx), cyclophosphamide given (Cy), testosterone treated (T) and antibursal serum administered (ABS). The mean values of the control and treatment groups for the different weeks are given in table 24.

Table 23. ANOVA table to find out the differences in total leucocyte count between 3 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	1.217593E+09	3.043983E+08	8.453814	**
Error	20	7.201445E+08	3.600722E+07		

** Significant ($P < 0.01$)

Table 24 Mean differen ial counts of non bursectomised and bursectomised ducklings at weekly intervals

Treatment	Age in weeks														
	1					2					3				
	L	H	E	B	M	L	H	E	B	M	L	H	E	B	M
Control	76 60	22 20	1 20		-	71 20	27 0	1 40	0 0		61 00	37 80	1 20		-
SBx	68 40	29 60	1 80	0 20	-	64 60	34 00	1 40		-	49 00	47 80	3 2		-
Cy	73 80	24 60	1 20		0 40	72 80	25 60	1 40		0 20	49 80	49 60	0 60		
T	71 40	27 00	1 40		0 20	62 40	37 00	0 60			63 80	35 60	0 60		
ABS	83 60	14 00	2 20	0 20	-	79 00	18 60	2 20	0 40	-	67 20	31 60	0 80	0 20	0 20

Treatment	Age in weeks														
	4					5					6				
	L	H	E	B	M	L	H	E	B	M	L	H	E	B	M
Control	56 00	43 40	0 60			54 60	43 80	1 40	0 20		76 40	22 20	1 0	0 20	
SBx	73 00	24 60	2 40			64 00	34 60	1 40			58 80	40 60	0 60		
Cy	64 40	35 00	0 40	0 20		68 00	31 60	0 40			66 40	30 60	3 00		
T	78 0	20 40	1 00	0 40		59 60	38 20	1 60	0 60		70 00	20 5	1 75		
ABS	53 60	45 20	1 20		-	66 60	32 20	1 00		0 20	62 00	36 40	1 40	0 20	

L = Lymphocyte
H = Heterophil
E = Eosinophil
B = Basophil
M = Monocyte

(contd)

Table 24 contd

Treatment	Age in weeks									
	7					8				
	L	H	E	B	M	L	H	E	B	M
Control	71 20	27 00	1 40	0 40		51 20	47 00	1 80		
SBx	53 60	43 40	2 40	0 40	0 20	58 40	41 20	0 40		
Cy	73 40	25 80	0 80			76 40	22 20	1 40		
T	79 25	18 00	2 75			49 25	48 50	2 25		
ABS	62 80	34 60	2 40	0 20	-	54 00	45 20	0 80		

Treatment	Age in weeks									
	9					10				
	L	H	E	B	M	L	H	E	B	M
Control	69 80	28 80	1 0		0 20	69 20	29 20	1 20	0 20	0 20
SBx	67 00	32 00	1 00			73 75	25 00	1 00		0 25
Cy	56 20	42 60	1 20			63 00	36 20	0 80		
T	71 25	25 50	3 00	0 25		68 50	28 75	2 50	0 25	
ABS	63 00	35 20	1 60	0 20		78 60	20 80	0 40	0 20	

L = Lymphocyte
H = Heterophil
E = Eosinophil
B = Basophil
M = Monocyte

Lymphocyte count

The mean per cent value of lymphocyte count in the control ducklings was highest in the first week (76.6%) and lowest in the eighth week (51.2%). In other weeks the values fall within this range.

Among the bursectomised ducklings, mean per cent value of lymphocyte was highest in ABS (83.6%) at week one and lowest in SBx group (49%) at third week.

In SBx group, the maximum count of lymphocytes was recorded at 10th week (73.75%) and the minimum at third week (49%).

Cyclophosphamide treated ducklings showed maximum percentage of lymphocytes (76.4%) at eighth week and minimum level (49.8%) at third week.

In testosterone administered ducklings the highest percentage of lymphocytes was seen at seventh week (79.25%) and the lowest percentage at eighth week (49.25%).

Antibursal serum given birds had highest count of lymphocytes at first week (83.6%) and lowest count at fourth week (53.6%) (Table 24).

Analysis of Variance of the lymphocyte counts at weekly intervals revealed significant differences between the control and treatments in the following cases.

At week one, no significant difference was noticed between

the control and treatment group, whereas significant difference ($P < 0.05$) was detected between lymphocyte counts of ABS (83.6%) and SBx (60.4%) and also between ABS and testosterone (71.4%) treated groups (Table 25).

Significant differences in lymphocyte counts after the first week was observed only in the fourth week. At the fourth week, significant differences in the lymphocyte count were recorded between the control (56%) and two treatments viz., SBx (73%) and testosterone (78.2%) treated groups. On comparison between the treatments, significant differences were observed ($P < 0.05$) between SBx (73%) and ABS (53.6%) and also between testosterone (78.2%) and ABS (53.6%) groups (Table 26).

After the fourth week, significant differences in the lymphocyte count was recorded only in the seventh week. In the seventh week, comparison of the control with treatment groups revealed significant differences ($P < 0.05$) only between control (71.2%) and SBx (53.6%). Comparing between the treatments, significant differences in lymphocyte counts ($P < 0.05$) were noticed between SBx (53.6%) and two treatment groups, viz., Cy (73.4%) and T (79.25%) (Table 27).

Significant differences in lymphocyte counts were also observed at the eighth week ($P < 0.05$) between control and treatment groups and also in between treatments. Control (51.2%) revealed significant difference, compared to Cy group (76.4%), while Cy group showed significant differences compared to SBx (58.4%), T (49.25%) and ABS (54%) (Table 28).

Table 25. ANOVA table to find out the differences in lymphocyte count between 1 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	306.086	76.52149	2.870845	*
Error	20	533.0938	26.65469		

* Significant ($P < 0.05$)

Table 26. ANOVA table to find out the differences in lymphocyte count between 4 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	903.6484	225.9121	4.172752	*
Error	20	1082.797	54.13984		

* Significant ($P < 0.05$)

Table 27. ANOVA table to find out the differences in lymphocyte count between 7 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	746.75	186.6875		
				3.18944	*
Error	19	1112.125	58.5329		

* Significant ($P < 0.05$)

Table 28. ANOVA table to find out the differences in lymphocyte count between 8 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	869.7382	217.4346		
				3.099508	*
Error	19	1332.875	70.15131		

* Significant ($P < 0.05$)

No significant difference in lymphocyte count between the control and treatment groups, or within treatments, was observed at the nineth and tenth weeks of age.

Heterophil count

The mean percent value of heterophil count in non-bursectomised (control) ducklings was highest (47%) in the eighth week and lowest (22.2%) at weeks one and six (Table 24).

Among the bursectomised ducklings, the highest count of heterophils was observed in Cy (49.6%) at the third week and the lowest count was seen in ABS (14%) at first week.

Surgically bursectomised ducklings revealed a maximum heterophil count at the third week (47.8%) and a minimum at fourth week (24.6%).

In Cy treated ducklings, a maximum heterophil count was observed at the third week (49.6%) and the minimum count at eighth week (22.2%).

Testosterone administered ducklings recorded the highest count of heterophils at the eighth week (48.5%) and the lowest count at seventh week (10%).

Among the ABS inoculated ducklings, the maximum heterophil count was observed at the fourth and eighth weeks (45.2%) and the minimum at first week (14%).

Analysis of Variance of the heterophil counts at weekly intervals revealed significant differences between the control

and treatments and between treatments, in the following cases:

At one week of age, no significant difference was observed between the heterophil counts of control and treatment groups, while significant differences ($P<0.05$) were observed in the counts of ABS (14%), when compared to SBx (29.6%), Cy (24.6%) and T (27%) treatments (Table 29). In the second week also, significant differences ($P<0.05$) were detected in the heterophil counts of ABS (18.6%), compared to SBx (34%) and T (37%) groups. No significant differences were noticed between other groups of the same age (Table 30).

Significant differences in heterophil counts after the second week were observed in week four. Control (43.4%) and the treatments (SBx (24.6%) and T (20.4%) showed significant differences ($P<0.01$). Comparing within treatments, significant differences ($P<0.01$) were seen between the following treatments: T (20.4%) compared to Cy (35%) and ABS (45.2%) and SBx (24.6%) compared to ABS (45.2%) (Table 31).

After the fourth week, significant differences in heterophil counts were noticed only at the seventh week. Control (27%) revealed significant differences ($P<0.05$) compared to SBx group (43.4%) only. Comparing the treated groups, significant differences in heterophil count ($P<0.05$) were observed between SBx (43.4%) and two treated groups, viz., Cy (25.8%) and T (18%). Significant differences ($P<0.05$) were also observed between T (18%) and ABS (34.6%) groups (Table 32).

Table 29. ANOVA table to find out the differences in heterophil count between 1 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	347.1406	86.78516	3.022279	*
Error	20	574.3028	28.71514		

* Significant ($P < 0.05$)

Table 30. ANOVA table to find out the differences in heterophil count between 2 week-old non-bursectorised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	434.6856	108.6714	3.086605	*
Error	20	704.1485	35.20742		

* Significant ($P < 0.05$)

Table 31. ANOVA table to find out the differences in heterophil count between 4 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	994.584	248.646		
				4.74941	**
Error	20	1047.061	52.35303		

** Significant ($P < 0.01$)

Table 32. ANOVA table to find out the differences in heterophil count between 7 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	720.0116	180.0029		
				3.144331	*
Error	19	1087.69	57.24692		

* Significant ($P < 0.05$)

In the eighth week also, significant heterophil count differences ($P < 0.05$) were noticed between control and treated groups and also in between treated groups. Control (47 %) showed significant difference only when compared to Cy (22.2%) group. Within the treated groups significant differences were observed between Cy (22.2%) and all other groups, viz., SBx (41.2%), T (48.5%) and ABS (45.2%) (Table 33).

No significant differences in heterophil counts were noticed at weeks nine and ten, between the control and treatments and also within treatments.

Eosinophil count

The mean value of eosinophil count in percentage, was highest in control ducklings at eighth week of age (1.8 %) and lowest at fourth week of age (0.6%) (Table 24).

Among the bursectomised ducklings the maximum eosinophil count was observed at the third week in SBx group (3.2%), and the minimum (0.4 %) was seen at weeks four and five in Cy, week eight in SBx and week ten in ABS groups (Table 24).

In the SBx group, the highest count of eosinophils was obtained at the third week (3.2%) and the lowest at eighth week (0.4%).

Cyclophosphamide administered ducklings gave a maximum eosinophil count at the sixth week (3%) and a minimum count at the fourth and fifth weeks (0.4%).

Table 33. ANOVA table to find out the differences in heterophil count between 8 week-old non-bursectomised and bursectomised ducklings

Source	DF	SS	MS	F	Inference
Treatments	4	868.8281	217.207	2.90598	*
Error	19	1420.152	74.74486		

* Significant ($P < 0.05$)

Testosterone given ducklings had maximum count of eosinophils at week nine (3%) and minimum count at weeks two and three (0.6%).

In ABS treated birds, the highest level of eosinophils was seen at seventh week (2.4%) and the lowest level at tenth week (0.4%).

Basophil count

In the control ducklings a basophil count of 0.2% was obtained in second, fifth, sixth and tenth weeks. In seventh week, a count of 0.4% was seen. The control birds did not give any basophilic counts at weeks one, three, four, eight and nine (Table 24).

Surgically bursectomised group of ducklings recorded a basophilic count of 0.2% in the first week, while in seventh week a count of 0.4% was obtained. All other weeks failed to give any counts.

Cyclophosphamide treated birds had a basophilic count of 0.2% in the fourth week only.

In testosterone given ducklings, a high basophil count of 0.6% was observed in week five, followed by 0.4% in week four and 0.25% in weeks nine and ten. No basophilic counts were obtained for the other weeks.

Antibursal serum administered ducklings showed a basophilic count of 0.2% in weeks one to three, six, seven, nine and ten.

Monocyte count

In the control ducklings, a monocyte count of 0.2% was obtained only in the ninath and tenth weeks (Table 24). In surgically bursectorised birds, a count of 0.2% was observed in seventh week and 0.25% in tenth week. Cyclophosphamide treated ducklings revealed a count of 0.4% in week one and 0.2% in week two. Testosterone administered ducklings had a monocyte count of 0.2% at week one only. In antibursal serum treated ducklings, a monocyte count of 0.2% was observed at weeks three and five only.

Discussion

DISCUSSION

Functioning of the immune mechanism is dependent on the lymphatic system. Avian lymphatic system is functionally divided into bursa-dependent and thymus-dependent components, of which the bursal component is associated with humoral immunity and the thymic component for cellular immunity (Firth, 1977). The bursa of Fabricius, a unique organ present in avian species, function centrally as the progenitor of immunocompetent cells in the humoral immune system, producing antibody molecules - the immunoglobulins. A study on the different classes of immunoglobulins and their characteristics is essential to know their importance in humoral immunity and significance in the pathogenesis of infectious diseases.

Ducks have two pairs of true lymph nodes and are hence considered as ideal experimental material for determining phylogenic immunological relations between mammals and birds. The susceptibility pattern of ducks to infectious diseases is quite different from that of chicken and they are generally more resistant to common avian diseases.

The role of bursa in antibody production was assessed by determining the antibody titres and quantitation of immunoglobulins in sera of non-bursectomised and bursectomised ducklings inoculated with SRBC/*S. typhimurium*.

Effect of bursectomy on the body weight

Non-bursectomised uninoculated ducklings had a mean body weight of 559.375 g at the fifth week of age, which showed a steady increase to 865 g at eighth week and 1132.50 g at 10th week. Hachimoto and Sugimura (1976) also recorded a rise in mean body weight of White Pekin ducks from day of hatching to 11 weeks of age. In non-bursectomised ducklings inoculated with SRBC/*S. typhimurium* also the body weights showed increase from fifth to tenth week. But in *S. typhimurium* given ducklings at eighth week, the body weights were significantly lower with $P < 0.05$ (751.875g), compared to SRBC inoculated (892.50 g) or uninoculated (865 g) ducklings. This difference however was not detected at the tenth week.

In surgically bursectomised ducklings, there were no significant differences in body weight between the non-bursectomised uninoculated control, SBxC, SBxSR and SBxSt. These results are in accordance with the observations of Mueller *et al.* (1963) who found that SBx had no effect on body weight. Heller and Derek (1973) also noted that SBx did not interfere with growth, eventhough a substantially lower weight gain occurred at three weeks following bursectomy.

Cyclophosphamide administered ducklings showed significant differences ($P < 0.01$) in body weights only at the eighth

week of age. Statistically significant reduction in mean body weights was observed in CyC (599.125 g), CySR (1055 g) and CySt (1109.167 g), when compared with the control (1240 g). The mean body weight of CyC group was also highly reduced, compared to CySR and CySt. Significant decrease in body weight of Cy-treated ducklings was observed from 7 days to 3 weeks (Hirase et al., 1973) and at one week of age (Hashimoto and Sugimura, 1976). They also observed that there were no differences in body weight between control and Cy-groups, after five weeks of age, and that at 7 weeks of age, the body weight of Cy-ducklings was even greater than that of the control. The results obtained in the present study revealed a statistically significant decrease in body weight at the 8th week, while no such differences were observed at fifth or tenth weeks. One possible reason for this disparity with earlier results could be that in this study a dose of 2.5 mg Cy was used while earlier workers have employed higher doses of 5-18 mg. Hence the suppressive effect of Cy might have become apparent only after five weeks and it might have worn off by ten weeks.

Ducklings hatched from eggs dipped in one per cent testosterone showed significant differences ($P < 0.05$) in mean body weight between the control (777.5 g) and two treatment groups, viz., TC (915 g) and TSR (961 g), at 10th week only. There was no significant difference in body weight between control and TSt group. Mueller et al. (1960) had reported that the

BBx using 19-nortestosterone produced birds weighing less than the controls. Glick (1963) also recorded a significant reduction in body weight at four weeks of age in two per cent TP treated ducklings, while no significant differences were found at the day-old stage. Contrary to these reports, in this study, the mean body weight instead of showing decrease, showed an increase from control in TC and TSR at 10th week. This could be due to the use of T instead of TP as it was done by others. Testosterone is easily metabolized and eliminated from the body without producing much effect on the bursal system. Eventhough TSt also showed increased body weight than control at tenth week, it was not statistically significant.

Antibursal serum administered ducklings revealed significant differences ($P < 0.05$) in body weight compared to control, only at eighth and tenth weeks of age. At eighth week, significant reduction in mean body weight occurred in ABC (704.167 g), ABSR (624.167 g) and ABSt (696.667 g), when compared to control (843.33 g). At the tenth week, however, ABSR had a significantly higher body weight (976 g) in comparison with control (707.5 g) and ABC (739 g). There are no published reports on the effect of antibursal serum in ducklings.

Among the different methods of bursectomy employed, cyclophosphamide was found to produce the maximum reduction in body weight compared to other treatments.

Effect of bursectomy on the weight of bursa

The mean bursal weight in uninoculated non-bursectomised ducklings showed a steady increase from the fifth week (0.955 g) to tenth week (1.175 g). The mean percentage of bursal weight in relation to body weight was maximum at fifth week (1.17%). In non-bursectomised ducklings inoculated with SNDC also a steady increase in mean bursal weight from 0.872 g (at 5th week) to 1.080 g (at 10th week) was noted, while in non-bursectomised, S. typhimurium given ducklings, the mean bursal weight increased from 0.961 g at fifth week to 0.998 g at eighth week and then again decreased to 0.930 g at tenth week. But statistical analysis of the data did not reveal any significant differences in weight of bursa between the uninoculated and inoculated groups of ducklings, at all the three age groups (5th, 8th and 10th weeks) under study. The percentage of bursal weight in relation to body weight was maximum in both above groups, at fifth week. Hashimoto and Sugimura (1976) have reported that the percentage of bursal weight in relation to body weight in White Pekin ducklings was maximum (0.24%) at one week of age after which it declined.

Surgically bursectomised ducklings lacked bursa completely, in all three age groups under study, thereby indicating the effectivsness of this method for bursectomy.

In Cy treated ducklings, significant reduction in bursal size, occurred at fifth, eighth and tenth weeks of age,

compared to control. At fifth week, the mean bursal weights of CyC (0.393 g), CySR (0.457 g) and CySt (0.396 g) were significantly different ($P < 0.01$) from that of control (0.959 g). Differences in the weight of bursa at the eighth week, between the control (1.039 g) and other groups of CyC (0.314 g), CySR (0.492 g) and CySt (0.577 g) were also statistically significant ($P < 0.01$). Significant differences in bursal weight ($P < 0.05$) were also observed at tenth week, between the control (0.709 g) and two treatment groups of CySR (0.249 g) and CySt (0.193 g). The bursal weights of CySR and CySt were also significantly smaller compared to CyC (0.783 g). Hiraga et al. (1976) found that the relative weight of the bursa in chicken decreased abruptly just after the injection of Cy and that it did not return to the control level until seven weeks of age. Significant reduction of bursal weight of Cy-treated ducklings have also been reported (Hashimoto and Sugimura, 1976; Sugimura et al.,1977).

Ducklings hatched out from testosterone treated eggs did not reveal any significant differences in bursal weight compared to the control group, in all three age groups of fifth, eighth and tenth weeks, under study. This result is contradictory to that obtained by earlier workers. Glick and Sadler (1961) found that dipping fertile eggs into solutions containing TP caused significant reduction of bursa size and that in some cases even eliminated the bursa. Administration of 19-nortestosterone or 17-ethyl-19-nortestosterone

on 12th or 13th DE caused a five to twenty-fold reduction in bursal weight (Mueller et al., 1962). Glick (1963) reported significantly reduced bursal size in ducklings hatched out from eggs which were dipped in 2 g % TP on the fifth day of incubation. In the present study, pure testosterone was used in 1% concentration, while other workers have used 19-nortestosterone or TP, in greater concentration. Testosterone was more easily metabolisable, compared to the other two derivatives and this might have reduced its antibursal effect at a faster rate, compared to the other two. Thus the lack of significant difference in the bursal weights of T-treated and control ducks could be attributed to the faster degradation and elimination of this hormone. The lower concentration used, to avoid mortality, could also be a contributory factor for the decreased effect.

Antibursal serum administered ducklings recorded significant differences ($P < 0.05$) in mean bursal weight between ABSR (0.519 g) and the control (0.870 g) and also between ABSR and ABC (1.050 g), at the eighth week only. Eventhough ABSt also showed decreased bursal weight (0.746 g) compared to control, it was not statistically significant.

On comparing the four methods of burosectomy, SBx had the maximum effect since burse was totally absent in all age groups. Cyclophosphamide produced marked reduction in burse size at fifth, eighth and tenth weeks. But CyC showed higher

bursal weight compared to control, at tenth week, indicating possible recovery. Among antibursal serum administered ducklings, reduction in bursal weight was noted only in antigen inoculated groups of ABSR and ABSt, compared to control, at 10th week, while ABC had higher bursal weight. This indicated that ABS had only a late action on bursa. The lack of significant difference in bursal weights between testosterone treated and control ducklings indicated that the effect of testosterone might have worn off very early even before hatching.

Effect of bursectomy on the weight of spleen

The mean splenic weights of non-bursectomised and uninoculated ducklings were 0.718 g at fifth week, 0.612 g at eighth week and 0.634 g at tenth week. Thus the splenic weight was maximum at fifth week. A successive increase in weight upto the 11th week as reported by Hashimoto and Sugimura (1977) was not seen here. It might be due to breed difference. Hashimoto and Sugimura (1977) used White Pekin ducks for their experiments, while cross-bred ducklings were used in the present experiment. The percentage of spleen in relation to body weight was also maximum (0.127%) at fifth week, while Hashimoto and Sugimura (1977) observed maximum percentage (0.2%) at third week. In STDC inoculated ducklings, the maximum mean splenic weight of 0.662 g was found at eighth week, while in S. typhimurium given group

it was seen at tenth week (0.790 g). Statistically there were no significant differences in splenic weights between uninoculated and inoculated birds at fifth, eighth and tenth weeks of age.

In surgically bursectomised ducklings, significant differences were noticed at eighth week ($P < 0.01$) in mean splenic weight between control (0.498 g) and the three treatments of SBxC (0.316 g), SBxSR (0.219 g) and SBxSt (0.279 g). But similar differences were not observed at fifth and tenth weeks of age. Similar decrease in spleen weight was observed by Heller and Perek (1973) at 5-6 weeks of age, in chicken which were surgically bursectomised at day-old stage. In the present study, SBx was performed at third day of age to reduce mortality.

Cyclophosphamide given ducklings revealed significant difference ($P < 0.01$) in weight of spleen at eighth week, between the control (0.806 g) and CyC (0.270 g) and also between CyC and the other two treatments of CySR (0.771 g) and CySt (0.641 g). No significant reduction in splenic weight was noted at fifth and tenth weeks. Rouse and Szenberg (1974) reported that Cy given on days 1, 2 and 4 post-hatching caused lymphocytic depletion of the bursa, thymus and spleen. Hashimoto and Sugimura (1976) observed significant reduction in weight of spleen of Cy-treated, newly hatched White Pekin ducklings, which lasted till seven

weeks of age. In this study, eventhough reduction in splenic weight was not seen in fifth or tenth weeks, significant reduction occurred in eighth week.

Testosterone administered ducklings, as in the case of body weight and bursal weight, failed to show any significant reduction in splenic weight also, compared to control, at all the three age groups of fifth, eighth and tenth weeks. Glick and Sadler (1961) observed that dipping fertile eggs into TP solution caused reduction in spleen size. Glick (1963) on the other hand reported no significant differences in the spleen weights of ducklings hatched from eggs dipped in TP solutions.

In antibursal serum given ducklings, no statistically significant differences were found in splenic weights at fifth, eighth or tenth weeks, between the control and treated groups. This indicated that ABS did not have any effect on spleen.

Among the four different methods of bursectomy employed in this study, surgical bursectomy resulted in significant reduction in spleen size of both surgically bursectomised uninoculated and inoculated ducklings, when compared to control group, at eighth week. Eventhough Cy-treatment also produced ducklings with reduced splenic weights at eight weeks of age, statistically significant reduction compared to control was seen only in uninoculated Cy-treated ducklings. Testosterone and ABS treatments did not cause any reduction in splenic size.

Histopathology

The role of bursa in the production of humoral immune responses was assessed by examining the histological features of the related lymphoid organs, viz., bursa and spleen, in non-bursectomised and bursectomised ducklings, at various age groups.

Bursa of Fabricius

In non-bursectomised ducklings given SRBC well defined follicles with loosely arranged lymphocytes and intact surface epithelium were seen at five weeks. Germinal centre activity and macrophage responses were seen at 8 weeks and by 10 weeks the lymphoid follicles were very active. *S. typhimurium* inoculated non-bursectomised ducklings revealed on the other hand, many active lymphoid follicles containing loosely arranged lymphoid cells even at five weeks. At eight weeks, the germinal centres of some of the active lymphoid follicles showed macrophage and histiocyte response and proliferation of macrophages occurred in perilymphoid locations. By ten weeks, many active lymphoid follicles had widened, active germinal centres with the lymphoid cells showing diffuse proliferation and there was also focal macrophage reaction in some areas. In the perilymphoid locations, reticular cell proliferation was also seen. Epithelial lining was intact.

In Cy-treated ducklings, there was extensive dwarfing

and thinning of the bursal folds, with severe degree of crypt formation. The number of follicles was very few with loosely arranged lymphoid cells. Epithelial lining was thrown into long, thin papillary folds in 8 week old bursa. Prasad (1978) found that administration of 4 mg of Cy per chick for three consecutive days post-hatching caused depletion of lymphoid cells in bursa and that the bursal lobes were reduced two to three times the normal size and remained so upto two weeks. Sachs et al. (1979) also recorded decreased plical size and thinning of follicles associated epithelium. The destruction of lymphoid cells in bursa of Cy-treated ducklings had been recorded by other workers also (Sugimura et al., 1974; Sugimura and Hashimoto, 1976). On inoculating Cy-treated birds with SRBC lymphoid follicles of bursa were found to be hypertrophic by the 8th week and active germinal centres were observed by 10th week, whereas stimulation with S. typhimurium produced active germinal centres by the eighth week itself. This indicated regeneration of the bursal follicles in response to antigenic stimulation. Glick (1971) also observed regeneration of some of the bursal follicles in chicks which had been given a single injection of Cy. In the present study also a single injection of Cy was given to ducklings and at a later stage, SRBC/S.typhimurium was also administered. Since no regenerative changes were detected in uninoculated Cy-treated birds, the changes noticed in this case, were due to antigenic stimulation.

Bursa of testosterone administered ducklings revealed along with initial degenerative changes of the epithelium, loosely arranged lymphoid cells throughout the period of observation. On the contrary, almost complete absence of lymphocytes in the bursa was reported in day old ducklings (Glick, 1963) and 19 day old chicken (Glick, 1969) after treatment with TP, although a regenerative change was observed in the latter by 43 days of age. But stimulation of bursal follicles characterized by activated germinal centres after inoculation with SRBC/S. typhimurium was observed in the present study and this reaction was profound in S. typhimurium given group during 10th week.

In the bursa of antibursal serum treated birds, the follicles were well formed, with loosely arranged lymphoid cells, but without active germinal centres at the 5th and the 8th weeks. When they were stimulated with SRBC, although initially there was a tendency for necrotic changes, by 8 weeks, diffuse lymphoid proliferation and by 10th week active germinal centre formation were observed. Inoculation with S. typhimurium also resulted in bursal stimulation with active germinal centre formation. There are no published reports on the effect of ABS on the histology of bursa.

On the whole, the bursal development was found to be highly suppressed on treatment with Cy. The ABS and testosterone treatments also elicited suppressive effect on bursa.

but to a comparatively, milder extent. On stimulation with SRBC/S. typhimurium, ABG as well as testosterone treated birds recorded bursal activation with germinal centre formation, but at a later stage in comparison with non-bursectomised birds. The Cy treated birds also reacted to antigenic stimulation at a later date, but with a comparatively weaker bursa having extensive atrophic changes.

The bursa has been considered as a central lymphoid organ in birds involved in the normal functioning of humoral immune system. But in the present study, it was observed that there was a profound proliferative change in the bursal follicles with active germinal centres indicating the organ's involvement in the immune response to antigenic stimulation. The macrophage as well as reticular cell reactions also was suggestive of a reacting bursa. Hence it appears reasonable to consider the bursa in ducks as an organ with probably a dual role, performing central as well as peripheral functions. This dual role had been observed in the bursa of chicken which formed specific antibodies against antigens introduced into the lumen, after its separation from the rest of the gut associated lymphoid tissue (Hippelainen et al., 1987).

Spleen

The spleen of non-bursectomised ducklings inoculated with SRBC revealed general proliferative lymphoid reaction, without follicle formation whereas S. typhimurium stimulated

ones revealed numerous follicles with active germinal centres by 10 weeks, along with the initial lymphoid proliferation. St. Pierre and Ackerman (1966) reported that in chicken, the development of lymphocytic nodules appeared to be dependent on the humoral action of the bursa. In the present study, the observation that only S. typhimurium (B dependent antigen) inoculated ducklings had spleen with numerous active follicles suggested that the above finding was true in the case of ducks also.

In surgically bursectomised ducklings, the lymphoid reaction in the spleen was not striking in any stage of observation. Stimulation with SRBC/S. typhimurium resulted in reticular cell hyperplasia, while diffuse proliferation of lymphocytes, especially in the periarterial sheath, was prominent in the latter case.

Cyclophosphamide treated birds revealed depletion of lymphoid cells in spleen, without follicle formation. Similar observation was made by Prasad (1978) in Cy-treated chicken. In addition, when SRBC was given in the present study to Cy-ducklings, diffuse lymphoid hyperplasia, a few microfollicles and a few germinal centres were observed. Active germinal centres were observed in the spleen from 5 week onwards when stimulated with S. typhimurium. This enhanced reaction also lends support to the postulation of St. Pierre and Ackerman (1966).

Glick (1969) reported that the number of bursa dependent follicles in the spleen was reduced in TP injected chicks. Active germinal centre formation was not observed in testosterone treated uninoculated ducklings in the present study also. But when these birds were stimulated with SRBC, reticular cell hyperplasia and active germinal centre formation by ten weeks were recorded. Similar but more extensive and striking reactions were observed in the case of S. typhimurium inoculated birds also, from five weeks onwards.

In antibursal serum treated ducklings, active splenic lymphoid follicles were observed by 10 weeks of age only, as against their occurrence by five weeks in SRBC/S. typhimurium stimulated groups.

From the present study, it was evident that bursa had a role in lymphoproliferative reactions of the spleen. In Sbx ducklings which completely lacked bursa, only mild lymphoproliferative change was observed, that too only in S. typhimurium inoculated group. Since the development of lymphocytic nodules in spleen was considered to be dependent on the humoral action of the bursa (St. Pierre and Ackerman, 1966) and since a bursa was absent in this case, the above reaction could have occurred with the help of B-cells derived from extrabursal sources.

In the present study, surgical Bx was conducted at 3 days of age. Glick (1963) had reported that the bursa of

ducks might release its immunologically competent cells or humoral substances during embryonic development. Hence the proliferative reaction seen in spleen could also have been due to such immunocompetent cells released from bursa during embryonic development.

The other groups of bursectomised ducklings possessed bursa, eventhough it was very much reduced in size in the Cy-group. In these cases the lympho-proliferative reaction was more marked. The increased intensity of reaction in these cases could be attributed to the existing and gradually regenerating bursa.

Separation and purification of serum globulins

Fractionation with neutral salts

Separation of globulins from pooled serum samples was carried out employing neutral salts like ammonium sulphate (at 33% and 40% levels) or sodium sulphate (in three stages of 18%, 14% and 14% final concentration). Higher protein concentrations were obtained for the globulins precipitated by 40% ammonium sulphate (5.969 g %) and by sodium sulphate (4 g %), compared to 33% ammonium sulphate (1.688 g %). But on immunoelectrophoretic analysis using antiduck serum raised in rabbit, the 33% ammonium sulphate precipitated fraction was found to be more pure, compared to the other two. Six precipitation arcs were produced by 33% ammonium sulphate precipitated globulin fraction against rabbit antiduck serum.

On the other hand 40% ammonium sulphate and sodium sulphate precipitated fractions had ten and twelve arcs of precipitation respectively, which was more similar to the immunoelectrophorogram of whole duck serum, where thirteen arcs were produced against antiduck serum. This indicated that 40% SAS and sodium sulphate did not completely remove the albumin fraction of serum and hence the higher protein concentrations obtained in these cases were due to contamination with albumin. This result was contradictory to the observation of Fei et al. (1986), who precipitated duck serum globulins using 40% SAS. The fractionation technique of Toth and Nagarcas (1981a) using sodium sulphate at two successive concentrations of 50% and 33% was not tried in this study. On further checking the purity of 33% ASS precipitated globulin and immunoelectrophoresis against rabbit antiduck globulin, two bold precipitation arcs were obtained, one extending from the well anodally (suggestive of IgM), and the other seen close to the antiserum trough and extending on either side of the antigen well (suggestive of IgG). Besides these, four faint arcs were also seen, merging with the above two, cathodally. Grey (1967a) related that immunoelectrophoresis of starch block isolated gamma-globulin from duck serum showed three antigenically distinct proteins in the gamma globulin region. One extended directly from the well, similar to mammalian gamma M globulins while the other two were located in the region where mammalian gamma G globulins were usually found.

Hence, of the three fractionation methods employed, 33% SAS precipitation was found to be more suitable for fractionation of duck globulins.

Purification of serum globulins by gel filtration chromatography

Ammonium sulphate precipitated globulins when subjected to Sephadex G-200 chromatography, two main peaks were revealed for the eluted globulin fractions. Fractionation of chicken serum globulins by this method by earlier workers also resulted in the elution of proteins in two main peaks, the first major peak being largely composed of IgM and alpha-2 macroglobulin and the second major peak composed of IgG (Higgins, 1976; Goel et al., 1980; Nandapalan et al., 1983). Grey (1967a) on the other hand obtained three elution peaks on sephadex G-200 gel filtration of duck gamma globulin fractions. The first peak was found to correspond to the IgM fraction, along with varying amounts of lipid and aggregated material, and two incompletely resolved peaks followed the first peak, representing the 7.0S and 5.7S IgG fractions respectively. Grey (1967a) also observed that the relative heights of the second and third peaks varied with the species of duck and state of immunization. The absence of a third peak in the present study might have been due to incomplete separation of IgG to 7.0S and 5.7S fractions. Eventhough the sephadex buffer used was the same, the height and width of the column was different in the two cases. A sephadex

column of 2.5 x 100 cm was used by Grey (1967a) while a shorter and thinner column of 1.5 x 70 cm was used in this study. Also, while Grey (1967a) used Muscovy, White Pekin and Mallard ducks for his experiment, cross-bred ducklings (Desi x Khaki Campbell) were employed for this study. This could also have contributed to the elimination of the third protein peak.

Immunoelectrophoresis of the pooled fractions of the ascending limb of the first major peak against rabbit anti-duck serum yielded a diffuse line extending anodally from the antigen well. According to the reports of the earlier workers mentioned above, the first major peak obtained after sephadex G-200 fractionation comprised of IgM and alpha-2 macroglobulin in chicken and IgM in ducks. Hence the fractions of the ascending limb of first major peak obtained in this study could be taken as IgM. The production of a diffuse line extending from the antigen well indicated that the IgM obtained was in semipure form. As IgA has not so far been detected in ducks (Parry and Aitken, 1975; Toth and Norcross, 1981a), the contaminant in IgM could be IgG. Concentrated and rerun ascending fractions of first major peak yielded on immunoelectrophoresis against specific hyperimmune serum, a single precipitation arc extending directly from the antigen well, anodally. These results are in accordance with the observation of Grey (1967a) who also found a precipitation arc extending directly from the well, similar to mammalian

gamma-M globulin, on immunoelectrophoresis of starch block isolated duck gammaglobulin. Hence the precipitation arc obtained in the present study on immunoelectrophoresis of rerun first peak fractions could be taken as that of purified IgM. Toth and Norcross (1981a) observed that duck IgM was an electrophoretically heterogenous protein with components migrating slower than IgM of other species and that the cathodal tip of duck IgM lines extended into the gamma-2 migration zone.

Fractions of the ascending limb of the second major peak when subjected to immunoelectrophoresis against rabbit anti-duck serum produced a precipitation arc located close to the antigen well and extending on either side of it. The same type of arc was produced against specific hyperimmune serum also, when concentrated and rerun second peak fractions were used. Immunoelectrophoresis of chicken IgG against its anti-IgG revealed that this arc corresponded with that produced by chicken IgG. Grey (1967b) revealed that duck 5.7 S protein formed a precipitin band very close to the antiserum trough, while the more antigenically complete and more slowly diffusing 7.8 S protein formed a band behind it. In immunoelectrophoresis using duck whole serum and its antiserum, Toth and Norcross (1981a) detected that besides the IgM and major IgG arcs, there occurred another arc also in the form of a thin line immediately within the curve of the major IgG line and merging with it towards the cathodal end. This was

presumed to be a minor IgG arc. But in this study such a minor arc was not found. It might be due to the formation of a single major peak, instead of two incompletely resolved peaks, following the first major peak. Even then, the production of a single precipitation arc by the second peak ascending fractions which corresponded with the arc produced by chicken IgG indicated that this arc was that of purified duck IgG. High cross-reactivity had been observed between chicken IgG and duck 7.8S Ig (Zimmerman et al., 1971; Hadge and Ambrosius, 1964). Hence the IgG which was obtained in the present study, could be 7.8 S Ig.

Serum protein

Total serum protein concentration in non-bursectomised and bursectomised ducklings

In non-bursectomised ducklings, the total serum protein was found to range from 1.813 g % (at first week) to 4.313 g% (at 8th week). This agreed with the reports of earlier workers (Brandt et al., 1951; Morgan and Glick, 1972). Defalco (1942) reported a value of 3.50 g % for total proteins in ducks, while Surendranathan (1966) obtained a total protein value of 4.85 ± 0.19 g % in adult male ducks and 5.00 ± 0.14 g % in adult nonlaying female.

Among the bursectomised ducklings, the ranges of total serum protein from weeks 1-8 were, 1.98-4.313 g % (SBx), 1.933-3.813 g % (Cy), 2.188-3.375 g % (T) and 3.0-2.313 g % (ABG). The serum protein levels in all four groups of

ducklings revealed a decrease in the second week, compared to the first week. But the control ducklings showed a steady increase in level. This might have been due to the effect of Bx. The effect might have been transient since from the third week, bursectomised ducklings of all groups except ABS recorded higher protein levels compared to second week. In ABS group, higher protein levels occurred from fourth week. The maximum serum protein level was observed at the 8th week of age in the control, SBx and testosterone treated groups, whereas in the Cy and ABS treated groups, the maximum levels were seen at the 7th and 5th weeks respectively. The serum protein levels at 10th week showed a decline in all the groups except ABS group. Morgan and Glick (1972) reported that neither surgical nor hormonal Bx had any significant effect on the total protein level in chicken. No published data are available on the effect of bursectomy in the serum protein level of ducks.

Total serum protein in non-bursectomised and bursectomised ducklings inoculated with SRBC/S. typhimurium

It was seen from the results (Table 13 and Fig. 3-6) that the total serum proteins of ducklings subjected to various treatments were not giving a regular pattern. But in general, there was an initial decrease, followed by an increase, a week later. Since similar work was not seen in published reports, these observations could not be compared. The initial decrease in serum protein might be due to the

immediate effect of antigen administration from which the birds recovered a week later as indicated by the rise in protein level.

Serological tests

a) Bacterial agglutination to detect antibody against S. typhimurium

Among bursectomised ducklings, antigen inoculation at all three age groups of 7, 28 and 42 days produced antibody titres far below those of controls. No antibody titres were produced in the following treatments. SBx group I (at day 7), group III (at day 7), Cy group I (at days 21 and 28), group II (at day 28) and group III (at days 7 to 28); Testosterone treated group I (at day 7), and group III (at day 14), and ABS group I and III (at day 7).

Glick et al. (1956) found that bursa played a vital role in the production of antibodies to S. typhimurium and that the antibody production was greatly decreased in birds bursectomised during the first few weeks after hatching. The effect was found to decrease with increase in age. Similar results were obtained in the present study in SBx birds in groups I and III wherein eventhough no titre was observed at day 7, increased titres were seen from day 14-28. Glick (1962) on the other hand had reported that Bx of White Pekin ducklings at four days of age or later, only slightly reduced the antibody response at six weeks of age. Contrary to this finding in this study the antibody level of SBx birds

given antigen even at 42 days was completely absent at 7 days post-inoculation.

St. Pierre and Ackerman (1965) recorded that chicks bursectomised by TP injection on the fifth day of incubation showed a marked inability to produce antibodies to S. typhimurium. In this study, a marked reduction in antibody titre was not seen in testosterone group, compared to other treatments, eventhough compared to control the reduction was marked. This can be attributed to the poor action of testosterone on the bursa, as discussed earlier.

Lerman and Weidanz (1970) found that treatment of chicks with 4-6 mg of Cy for first three days of life suppressed primary and secondary responses to S. typhimurium. The results obtained with Cy-group in this study are in agreement with this observation.

No data are available on the antibody titre in birds bursectomised by ABS. But a reduction in titre compared to control was seen in ABS group also, indicating suppression of specific immune response. This observation is quite interesting from the point of view that ABS did not have much effect on the bursal weight and in its histopathological appearance. Probably, ABS specifically acted on B cells concerned with recognition of S. typhimurium antigen.

b) Sheep red blood cell agglutination

Among the bursectomised ducklings, lowered antibody

titres than the control were observed in all three antigen given groups of SBx, Cy and T and in groups II and III of ABS treated birds. Group I ABS administered ducklings revealed titres identical to that of the control at days 7, 14 and 21 post-inoculation, while at day 28 the titre was lower.

The results revealed that in SBx and T treatment groups, inoculation of antigen at days 7 and 28 produced steadily increasing levels of antibody, while administration of antigen by day 42 produced identical antibody levels in all four weeks of observation in SBx, and steadily decreasing titres in T-group. In Cy treated ducklings, antigen administration at 42nd day revealed a very low antibody titre at first week post-inoculation and at other weeks titres were completely absent. In groups I and II titres showed an increase upto day 14 and then decreased ABS given birds had steadily increasing titres of antibody by inoculation of antigen at days 7 or 42, while antigen inoculation at day 28 produced steadily decreasing titres.

Glick et al. (1956) found that in chicken bursectomised during the first few weeks after hatching antibody production against SRBC was reduced. Glick (1962) reported that Bx of White Pekin ducklings at four days of age or later, only slightly reduced the antibody response at six weeks of age and that at 24 weeks, bursectomised birds exhibited a significantly lower antibody titre than controls. Eventhough the

antibody response of the White Pekin duck was not influenced by SBx at hatching, the ducklings hatched out from eggs dipped in 2 g % TP on 5th day of incubation failed to respond to Salmonella pullorum (Glick, 1963). Hirota and Dito (1975) found that antibodies produced by TP-treated chicken against SRBC were almost exclusively IgM type, and that hormonally bursectomised chicken responded to SRBC to a higher extent than those surgically bursectomised in a newly hatched period. Hirota et al. (1976) reported that the production of IgM antibody against SRBC was not affected significantly by TP, while immune responses against bacterial antigens and the production of IgG antibodies were strongly suppressed.

Lerman and Weidenz (1970) observed that treatment of chicks with 4-6 mg of Cy for first 3 days of life suppressed the primary and secondary responses to SRBC.

Contradictory to the observation of Glick (1962) in this study SBx of ducklings produced suppression of anti-SRBC titres even from day 7 post-inoculation. While Glick (1962) performed Bx at 4 days of age, in this case Bx was done at 3 days of age, which might contribute to some extent to the reduced titres obtained. At the same time, reduced antibody titre in ducklings hatched from testosterone dipped eggs eventhough was seen at 7th day in group I, it steadily increased to 128, contrary to the observation of Glick (1963). It might be due to the use of testosterone in this study, instead of TP, at a lower concentration of 1 g %. The severe

suppression of antibody responses in groups I and III of Cy-treated ducklings was in agreement with the observations of Lerman and Weidanz (1970). The higher antibody titres obtained in testosterone treated group, compared to SBx group, was in accordance with the findings of Hirota and Sito (1975). Suppression of antibody titres was also seen in ABS given ducklings, in groups II and III while in group I the titres were more or less similar to that of the control. These identical antibody titres as that of the control seen in group I might have been due to IgM antibodies, since Nakatani et al. (1986) have reported that the IgM antibody responses were found to be relatively insusceptible to the cytotoxic effect of ABS while IgG responses were highly susceptible. There are no published reports on the effect of ABS in ducklings.

Quantification of immunoglobulins

Concentration of IgM in serum of non-bursectomised and bursectomised ducklings

Among bursectomised ducklings, SBx group showed a higher IgM level than age-matched control at weeks 5, 6, 9 and 10. Cyclophosphamide treated ducklings recorded higher IgM levels than age-matched control at weeks 3-7 while in testosterone given ducklings higher than control IgM levels were observed at weeks 3 to 6, 9 and 10. In antibursal serum administered birds, the IgM levels were higher than age-matched controls at weeks 6, 9 and 10. The serum IgM levels of the control

and all the bursectomised groups except ABS group, were high in the first week and thereafter decreased. In ABS group there was a gradual rise in IgM concentration from the first to the third week.

Many workers have observed elevated IgM levels in bursectomised chicken. But similar data are not available for ducks. Claflin et al. (1966) reported that surgically or hormonally bursectomised chicken synthesized chiefly IgM type antibodies. Van Meter et al. (1969) have reported that despite its suppressive effect on primary antibody responses, Bx at hatching had no effect on the early rise in circulating IgM. Lerner et al. (1971) also observed that IgM levels were markedly elevated with earlier Bx. Morgan and Glick (1972) recorded higher than control IgM levels in SBx and MBx groups. Sawada and Bito (1980) found that cells producing IgM antibodies were less susceptible to the cytotoxic effect of antibursal serum, compared to those producing IgG antibodies. The elevated IgM levels obtained in treated ducklings in this study are in accordance with the results of the above workers.

Lerman and Weidanz (1970) observed that 4-6 mg of Cy given for first three consecutive days of life reduced IgM and IgG levels in chicks. But in the present study, Cy treated ducklings had elevated IgM levels compared to control at weeks 3-7. This might have been due to the lower dose (2.5 mg) of Cy used.

Serum concentration of IgM in non-bursectomised and bursectomised ducklings inoculated with SRBC/S.typhimurium

Comparing the three age groups at which SRBC was administered in bursectomised ducklings, IgM values higher than control level were obtained in the following cases: In SBx SR group I and group II; in CySR group II and Group III; in TSR group I and group II; in ABSR group I, group II and group III.

S. typhimurium inoculated bursectomised ducklings revealed in the following treatments, higher IgM levels than control at the three age groups, on antigen inoculation. In SBxSt higher levels were seen in groups I and II. Higher levels of IgM were obtained in CySt groups I to III; in TSt group I and in ABSt group II and group III.

The increased IgM levels than control seen in bursectomised inoculated ducklings corresponded to earlier reports of elevated IgM levels in bursectomised birds (Claflin et al., 1966; Van Meter et al., 1969; Lerner et al., 1971; Morgan and Glick, 1972; Sawada and Bito, 1980).

Serum concentration of IgG in non-bursectomised and bursectomised ducklings

Among the bursectomised ducklings, in SBx group, lower IgG levels than control were obtained in weeks 1-4, 7, 8 and 10 while the levels were higher at other weeks. In Cy treated group, weeks 1-4 and 7 showed lower IgG levels while at weeks 5 and 6 the levels were identical and at weeks 8-10

the levels were higher than control. Testosterone administered ducklings revealed lower IgG levels than control at weeks 1-4, 7 and 8. Higher IgG levels were detected at weeks 5, 9 and 10, while at week 6 level identical to control was obtained. In ABS treated group, lower IgG levels were obtained at weeks 1, 2 and 4 compared to control. At 8th week the IgG levels of both ABS and control groups were the same, while the levels for ABS were higher than control at weeks 3, 5 to 7 and 9 and 10.

Van Meter et al. (1969) reported that Bx at hatching delayed, but did not prevent the normal increase with age of plasma IgG concentration. Morgan and Glick (1972) also observed a delay in normal IgG production in chicken bursectomised surgically or hormonally. Peray and Diamenstock (1973) found that Bx at hatching led to a moderate decrease in IgG, compared to sham bursectomised chicken. Hirota and Bito (1975) observed decreased level of IgG until 8th week in bursectomised chicken.

Lerner et al. (1971) reported that Bx performed as early as the 3 DE with testosterone propionate resulted in marked lowering of IgG.

Lerman and Weidanz (1970) detected reduced levels of IgG in chicken treated with 4-6 mg of Cy in the first 3 days of life. Cells producing IgG antibodies were found to be highly susceptible to antibursal serum (Sawada and Bito, 1980; Nakatani et al., 1986).

There are no published reports on the IgG levels in bursectomised ducks. But the reduced IgG levels at the first four weeks in case of SBx, Cy and T-groups are in accordance with the previous reports on IgG levels in bursectomised chicken. In ABS group, reduction in IgG level was seen at 1, 2 and 4 weeks only, indicating a lesser suppressive effect by ABS on bursa, compared to the suppression produced by other treatments.

Serum concentration of IgG in non-bursectomised and bursectomised ducklings inoculated with SRBC/*S. typhimurium*

Among bursectomised ducklings given SRBC, SBxSR showed higher IgG levels than control in group II and groupIII. In CySR, higher IgG levels compared to control were recorded in group II and group III. Testosterone administered and SRBC given ducklings revealed IgG values which were higher than control in group II and group III. ABS treated ducklings had higher IgG concentration in comparison with control in group II and in group III. In group I, the treated ducklings revealed either lower or identical IgG levels compared to age-matched controls.

In *S. typhimurium* inoculated bursectomised ducklings, higher IgG levels than that of age-matched control were observed in groups I to III of SBxSt and CySt; in groups II and III of TSt, and in groups I to III of ABSt.

The production of lower levels of IgG than control in SRBC given bursectomised ducklings of group I was in

accordance with the findings of other workers (Van Meter et al., 1969; Lerman and Weidana, 1970; Lerner et al., 1971; Morgan and Glick, 1972; Percy and Dienenstock, 1973; Hirota and Bito, 1975; Sawada and Bito, 1980; Nakatani et al., 1986). At the same time, higher IgG levels compared to control, seen in groups II and III of SRBC given and all three groups of S. typhimurium given bursectomised ducklings are contradictory to these findings. Glick (1986) observed that while Cy-treated birds lacked IgG antibody to SRBC, about 50 % of the Cy birds produced non-specific IgG. Such non-specific IgG production might be the cause of elevated IgG levels in bursectomised birds of this study also.

Concentration of immunoglobulins in bile and egg yolk

On quantifying the immunoglobulin in bile of non-bursectomised and bursectomised ducklings, precipitation rings were produced only against anti-IgM, while no ring formation occurred against anti-IgG. This indicated the presence of only IgM in bile of ducklings. Immunoelectrophoresis of duck bile against antiduck serum also confirmed this observation. These results are in agreement with the observations of Pamela and Higgins (1986) who found Ig of a single class in duck bile, with a molecular weight of 890,000 and suggested that duck biliary Ig was an IgM-like molecule secreted independently of serum Ig. Studies by Hadge and Ambrosius (1988b) also revealed that the biliary immunoglobulins of anseriform birds were IgM-like.

While the bile of control ducklings showed a high concentration of IgM (4.025 mg/ml), the IgM concentration was greatly reduced in BSx (2.0 mg/ml), Cy (1.0 mg/ml), T (2.75 mg/ml) and ABS (3.025 mg/ml) groups. While no published reports are available giving IgM values in bile of bursectomised ducks, Hadge and Ambrosius (1988a) in a comparative study on the structure of biliary immunoglobulins from chicken, turkey, duck and goose, had observed that the bile from these birds contained immunoglobulins in relatively high amounts of 4.5 to 15 mg/ml. The decrease in IgM concentration in bile following bursectomy and the absence of such an effect in serum concentration of IgM in the same group of birds indicated that in the former case, the cells concerned with IgM production were dependent on bursa, but not so in the latter.

When the immunoglobulins in egg yolk samples were quantitated by Mancini's method precipitation rings were produced against anti-IgM and anti-IgG, indicating the presence of both IgM and IgG in egg yolk. Eventhough the quantitation of egg yolk immunoglobulins in ducks had not been done so far, the above findings were in agreement with the observations of Yamamoto et al. (1975) who found IgM and IgA also, besides IgG in concentrated preparations of egg yolk of chicken.

In this study, the IgM level in egg yolk was found to be

in the range of 2.82 mg/ml to 4.44 mg/ml, with a mean concentration of 3.425 mg/ml. The IgG concentration ranged from 7.18 mg/ml to 9.64 mg/ml, with a mean value of 8.0 mg/ml. Only the quantitation of IgG in egg yolk had been attempted previously, that too only in the domestic fowl and in the turkey. The amounts of IgG in yolk had been reported to be 20-25 mg/ml in hen's egg and 2-6 mg/ml in turkey's egg (Rose and Orlans, 1981).

Role of bursa in antibody production

The results obtained in bacterial agglutination and SRBC agglutination tests indicated that an intact bursa was required for specific antibody production in ducks. This observation was contradictory to that of Glick (1963), who found that while Bx of White Pekin ducklings at 4 days of age or later only slightly reduced the antibody response at 6 weeks, a significantly lower antibody titre than control was obtained at 24 weeks. This led Glick (1963) to postulate that Bx after hatching would be too late to significantly influence the antibody response of the duck as the bursa of ducks might release its immunologically competent cells or humoral substances during embryonic development.

The fact that the bursa-less bird was able to produce IgM^a, suggested that the function of the bursa was primarily to induce the transition from synthesis of IgM to that of IgG rather than to initiate IgM^a synthesis itself. Under

circumstances in which the bursa was prevented at an early stage from forming the potential to produce IgM might develop in another part of the body but the non-bursal site might not be able to induce efficient differentiation of the IgG system. These results indicated that the bursa had a major role only in the maturation from IgM to IgG synthesis (Lerner et al., 1971). Moticka and Van Alten (1972) have also suggested that bursa might not be necessary for all types of antibody responses. By experiments involving SBK from as early as 52 h of embryonation, Jankovic et al. (1975) also observed that the bursa might not be obligatory for the development of the bursal cell line.

Comparative studies of B-cell development in the bursa and bone marrow of the chicken after hatching provided support for the view that the bone marrow played an important role in the generation of the B-cell repertoire (Kincade et al., 1973). Moticka (1975) also postulated that the bone marrow could be a non-bursal site for B-cell differentiation. Glick and Rosse (1981) suggested that avian bone marrow might possess a progenitor pool for virgin B cells that was distinct from B cell progenitors in the bursa and was independent of that organ. Befus et al. (1980) on the other hand postulated that the widespread mucosal network comprising of the bronchial lymphoid aggregates, Harderian gland and gut associated lymphoid tissue might in addition to acting

a secondary lymphoid tissue, represent the bursal independent sites of B-cell differentiation in chicken.

The presence of elevated levels of IgM and IgG compared to control in bursectomised ducklings, in spite of a decrease in specific antibody production against SRBC/S. typhimurium indicated that as in the case of chickens, ducks might also possess extra-bursal B-cells independent of the bursa of Fabricius. Eventhough the site of production of these extra-bursal B-cells are not known till date, evidences are there to indicate that the lymph nodes in ducks may have a role in their production. There was no decrease of plasma cells in the lymph nodes nor in the spleens of bursectomised ducks (Sugimura and Hashimoto, 1976), in which antibody-producing capability to Salmonella pullorum was severely eliminated (Hashimoto and Sugimura, 1976). This finding showed that plasma cells might not originate from the bursa. Sugimura et al. (1977) observed that in duck lymph nodes, the para sinusoidal area of lymphatic nodules and the existence of plasma cells appeared to be independent of both the thymus and the bursa of Fabricius. The presence of higher IgG levels in bursectomised ducklings compared to the control indicated that the IgG produced might be non-specific. Similar non-specific IgG production had been reported in Cy-treated birds which lacked IgG antibody to SRBC (Glick, 1966).

Haematology

A comparison of week-wise total leucocyte counts revealed

statistically significant difference ($P<0.01$) only at the third week, that too only between the testosterone treated group and the SBx and Cy groups. Glick (1963) on the other hand did not find any alteration in WBC count after surgical or hormonal Dx. Jalkanen et al. (1983) also reported that SBx had no effect on the total lymphocyte and other white cell counts in the peripheral blood of chicken.

At many of the weeks under study, control and bursectomised ducklings revealed total leucocyte counts which were higher than that observed by earlier workers (Magath and Higgins, 1934; Sreenivasan and Rao, 1965; Surendranathan, 1966). The reasons for this might be breed difference and altered technique employed.

Significant differences in lymphocyte count between the control and various treatment groups, under study were detected only at weeks 4, 7 and 8. Significantly higher lymphocyte count compared to the control was seen at the fourth week (in SBx and T) and at the eighth week (in Cy) while significantly lower count than control was obtained by SBx at the 7th week.

Administration of testosterone propionate had no significant influence on the absolute lymphocyte count of ducklings and chicks (Glick, 1963; 1969). Cyclophosphamide was also not found to change the absolute number of lymphocytes (Glick, 1986). But antibursal globulin administration produced a fall in circulating lymphocytes of chicks

(Jankovic et al., 1970). The results obtained in the present study were contradictory to these observations.

Statistically significant differences in heterophil counts were observed between the control and bursectomised ducklings in the following cases. At the fourth week SBx and T groups had significantly lowered heterophil counts, compared to control. SBx group showed significantly higher heterophil count than the control at the 7th week, while at the 8th week, Cy-treated birds had markedly lower count, compared to the control.

Glick (1963) observed that the percentage of heterophil count in 4 week-old ducklings was not significantly influenced by SBx. Similar results were obtained in the present study also. The absolute number of granulocytes was not changed by Cy-treatment of chicken (Glick, 1986).

Jankovic et al. (1970) reported that antibursal globulin administration produced a fall in granulocyte counts of peripheral blood in chicks. Similar results were not observed in the present study.

Compared to the control, eosinophil counts in the bursectomised ducklings were higher, while the basophil and monocyte counts in control and treated groups were more or less the same. The basophil and monocyte counts obtained in this study were much lower than that obtained by previous workers (Nagath and Higgins, 1934; Sreenivasan and Rao, 1965; Surendranathan, 1966). This might be due to breed difference.

Summary

SUMMARY

The immunoglobulin profile and the role of bursa in antibody production in ducks were investigated and presented.

The role of bursa was assessed by determining the antibody titres and quantitation of immunoglobulins in the sera of non-bursectomised and bursectomised ducklings inoculated with SRBC/*S. typhimurium*. Bursectomy was performed by surgical, chemical, hormonal methods or by treatment with antibursal serum. Antigen administrations were done at 7, 28 or 42 days of age. The ducklings were sacrificed four weeks after inoculation, viz., at the 5th, 8th or 10th weeks of age and the body weights and weights of bursa and spleen determined. Histopathological studies on the bursa and spleen were also conducted. The total and differential leucocyte counts in non-bursectomised and bursectomised ducklings were also determined.

The immunoglobulin profile of normal ducks was studied by separation and purification of various classes of immunoglobulins in duck serum and by quantitation of the immunoglobulins in serum, bile and egg yolk.

In surgically bursectomised ducklings, there were no significant differences in body weight between the non-bursectomised uninoculated control, SBxC, SBxSR and SBxSt.

Cyclophosphamide administered ducklings showed statistically significant ($P < 0.01$) reduction in mean body weights in CyC (598.125 g), CySR (1055 g) and CySt (1109.167 g), when compared with the control (1240 g), at the eighth week of age only.

Ducklings hatched from eggs dipped in one per cent testosterone showed significant differences ($P < 0.05$) in mean body weight between the control (777.5 g) and two treatment groups, viz., TC (915 g) and TCR (961 g), at 10th week only.

Antibursal serum administered ducklings revealed significant reduction ($P < 0.05$) in mean body weight in ABC (704.167 g), ABSR (624.167 g) and ABSt (696.667 g), when compared to control (863.33 g) at the 8th week. At the 10th week, however, ABSR had a significantly higher body weight (976 g) in comparison with control (787.5 g) and ABC (739 g).

Among the different methods of bursectomy employed, Cy was found to produce the maximum reduction in body weight compared to other treatments.

Statistically significant differences were not found in weight of bursa between the uninoculated and inoculated groups of non-bursectomised ducklings, at 5th, 8th and 10th weeks.

Surgically bursectomised ducklings lacked bursa completely, in all three age groups under study.

In Cy treated ducklings, significant reduction in bursal size occurred at 5th, 8th ($P<0.01$) and 10th ($P<0.05$) weeks of age. At 10th week however, significant differences were observed only between the control (0.709 g) and two treatment groups of CySR (0.249 g) and CySt (0.195 g), while bursa of CyC had a higher weight (0.783 g) than the control.

No significant differences were found in bursal weight of testosterone treated ducklings in all three age groups under study.

Antibursal serum administered ducklings recorded significant differences ($P<0.05$) in mean bursal weight between ABSR (0.519 g) and the control (0.870 g) and also between ABSR and ABC (1.050 g) at the 8th week only.

On comparing the four methods of bursectomy, SBx was found to have the maximum effect since bursa was totally absent in all age groups under study.

Statistically there were no significant differences in splenic weight between uninoculated and inoculated non-bursectomised ducklings at 5th, 8th and 10th weeks of age.

In surgically bursectomised ducklings, significant differences were noticed in mean splenic weight ($P<0.01$) at the eighth week between control (0.498 g) and the three treatment groups of SBxC (0.316 g), SBxSR (0.219 g) and SBxSt (0.279 g).

Cyclophosphamide given ducklings revealed significant differences ($P < 0.01$) in weight of spleen at the eighth week, between the control (0.806 g) and CyC (0.270 g) and also between CyC and the other two groups of CySR (0.771 g) and CySt (0.641 g).

Testosterone and ABS administered ducklings also failed to show any significant reduction in splenic weight.

Among the four different methods of bursectomy employed in this study, SBx produced significant reduction in spleen size of both uninoculated and inoculated ducklings, at the eighth week.

Histopathological studies of the bursa revealed in CSR, at five weeks, well defined follicles with loosely arranged lymphocytes and intact surface epithelium. Germinal centre activity and macrophage responses were seen at 8 weeks and by 10 weeks the lymphoid follicles were very active. CSt ducklings revealed on the other hand, many active lymphoid follicles containing loosely arranged lymphoid cells even at five weeks and by ten weeks, many active lymphoid follicles had widened, active germinal centres.

Among the bursectomised ducklings, bursa was completely absent in SBx group. In Cy-treated ducklings, there was extensive dwarfing and thinning of the bursal folds, with severe degree of crypt formation. The number of follicles was very few, with loosely arranged lymphoid cells. Lymphoid

follicles of bursa were found to be hypertrophic by 8th week and active germinal centres were observed by 10th week in CySR ducklings. In CySt group active germinal centres were produced by the 8th week itself.

Bursa of testosterone administered ducklings revealed along with initial degenerative changes of the epithelium, loosely arranged lymphoid cells throughout the period of observation. Inoculation with SRBC/S. typhimurium resulted in activated germinal centres and this reaction was profound in S. typhimurium given group during 10th week.

In the burse of antibursal serum treated birds, the follicles were well formed, with loosely arranged lymphoid cells, and when stimulated with SRBC/S. typhimurium bursal stimulation occurred with active germinal centre formation.

The spleen of CSR ducklings revealed general proliferative lymphoid reaction, without follicle formation, whereas spleen of S. typhimurium stimulated birds showed numerous follicles with active germinal centres by 10 weeks, along with the initial lymphoid proliferation.

In SRx ducklings, stimulation with SRBC/S. typhimurium produced reticular cell hyperplasia, and diffuse proliferation of lymphocytes in spleen.

Cyclophosphamide treated birds revealed depletion of lymphoid cells in spleen, without follicle formation. When SRBC/S. typhimurium was given germinal centre formation was observed.

In testosterone treated ducklings, active germinal centre formation in spleen was observed only on stimulation with SRBC/S. typhimurium. In ABS group, active splenic lymphoid follicles were observed by 10 weeks of age only, as against their occurrence by five weeks in SRBC/S. typhimurium stimulated groups.

Ammonium sulphate at 33% level was found to be ideal for fractionation of duck serum globulins.

Immunoelectrophoresis of whole duck serum against anti-duck serum produced 13 precipitation arcs, while precipitated globulin showed six arcs against the same antiserum. On immunoelectrophoresis against antiduck globulin, the precipitated globulin revealed mainly two bold precipitation arcs, one extending from the well anodally (suggestive of IgM), and the other seen close to the antiserum trough and extending on either side of the antigen well (suggestive of IgG). Duck bile on immunoelectrophoresis against antiduck serum, produced a single precipitation arc, extending anodally from the well (suggestive of IgA).

Ammonium sulphate precipitated globulins when subjected to sephadex G-200 chromatography, two main peaks were revealed for the eluted globulin fractions. Concentrated and serum ascending fractions of first major peak yielded on immunoelectrophoresis against specific hyperimmune serum, a single precipitation arc extending directly from the antigen

well anodally and this formed purified IgM. A precipitation arc located close to the antigen well and extending on either side of it was detected on immunoelectrophoresis of concentrated and rerun second peak fractions against specific hyperimmune serum and it formed purified duck IgG. Immunoelectrophoresis of chicken IgG against its anti-IgG revealed that this arc corresponded with that produced by chicken IgG.

The total serum protein concentration in non-bursectomised ducklings was found to range from 1.813g% (at first week) to 4.313 g % (at 8th week). Among the bursectomised ducklings, the serum protein levels in all four groups revealed a decrease in the second week, compared to the first week. From the third week, bursectomised ducklings of all groups except ABS recorded higher protein levels compared to second week. In ABS group, higher protein levels occurred from fourth week. At 10th week, the serum protein levels showed a decline in all the groups except ABS group.

In non-bursectomised and bursectomised ducklings inoculated with SRBC/S. typhimurium, the total serum protein concentrations obtained were not consistent. Comparing the three age groups of SRBC inoculation, only SBxSR had lower than CSP total serum protein levels in groups I and II. In S. typhimurium inoculated, bursectomised ducklings, only SBxSt had serum protein levels lower than that of CSt in groups I and III.

Bacterial agglutination test revealed that in all four groups of bursectomised ducklings, antigen inoculation at 7, 28 or 42 days produced antibody titres far below those of controls.

In SRBC agglutination test, among bursectomised ducklings, lowered antibody titres than the control were observed in groups I to III of SBx, Cy and T treated and groups II and III of ABS treated birds. Group I ABS administered ducklings revealed titres identical to that of control at days 7, 14 and 21 post-inoculation, while at day 28 the titre was lower.

Bursectomised uninoculated ducklings revealed higher IgM level than age-matched control at weeks 5, 6, 9 and 10 (SBx); at weeks 3-7 (Cy); at weeks 3-6, 9 and 10 (T); and at weeks 2-6 and 10 (ABS). The serum IgM levels of the control and all the bursectomised groups except ABS group, were high in the first week and thereafter decreased.

In bursectomised ducklings administered SRBC, IgM values higher than control level were obtained in the following cases: in groups I and II of SBxSR and TSR; groups II and III of CySR; and groups I to III of ABSR. S. typhimurium inoculated bursectomised ducklings revealed in the following treatments, higher IgM levels than control: in SBxSt groups I and II; CySt groups I to III; TSt groups I; and in ABSt groups II and III.

Quantitation of IgG levels in bursectomised ducklings revealed that in SBx, Cy and T groups lowered IgG levels compared to age-matched control were obtained in weeks 1-4 of age. From weeks 5-10, the levels were higher, lower or the same as that of controls, in an inconsistent manner. In ABS treated group, compared to control, lower IgG levels were obtained at weeks 1, 2 and 4, while at 8th week the level was the same as that of the control and at other weeks, higher.

In bursectomised ducklings administered SRBC, higher IgG levels than control were obtained in groups II and III of SBxSP, CySR, TSR and ABSR. In group I, the treated ducklings revealed either lower or identical IgG levels compared to age-matched controls.

S. typhimurium inoculated bursectomised ducklings revealed higher IgG levels than that of age-matched control in groups I to III in SBxSt, CySt, TSt and ABSt.

Quantitation of immunoglobulins in bile revealed the presence of only IgM. The IgM level in the bile of bursectomised ducklings was lower than that of the age matched control.

Both IgM and IgG were found to be present in egg yolk of duck embryos. The mean concentrations of IgM and IgG were 3.425 mg/ml and 8.0 mg/ml respectively.

Significantly higher lymphocyte count between the control and various treatment groups under study were detected

only at weeks 4 (SBx and T groups), and 8 (Cy group). At 7th week, SBx group had significantly lower lymphocyte count, compared to control.

With regard to the heterophil count, at the fourth week of age SBx and T groups had significantly lowered counts, compared to control. SBx group showed significantly higher heterophil count than the control at the 7th week, while at the 8th week, Cy-treated birds had markedly lower count, compared to the control.

Compared to the control, eosinophil counts in the bursectomised ducklings were higher, while the basophil and monocyte counts in control and treated groups were more or less the same.

In brief, from the present study the following important conclusions were obtained.

1. Among the different methods of bursectomy employed, Cy was found to produce the maximum reduction in body weight, while SBx resulted in in toto absence of bursa and significant reduction of spleen size of both surgically bursectomised uninoculated and inoculated ducklings.
2. Histopathological studies revealed that bursa had a role in lymphoproliferative reactions of spleen. The maximum suppressive effect on bursa was produced by Cy, while maximum suppressive effect on spleen was seen in SBx group.

3. Ammonium sulphate precipitation (at 33% level) was found to be ideal for the separation of duck serum globulins.

4. Sephadex G-200 gel filtration was suitable for purification of IgM and IgG of ducks.

5. The production of very low agglutination titres against S. typhimurium and SRBC and the presence of elevated IgM and IgG levels in bursectomised ducklings inoculated SRBC/S. typhimurium indicated that bursa was concerned with only the production of specific antibodies and that extraburssal B-cells were responsible for production of non-specific immunoglobulins.

6. Bile of ducks contained IgA, as evidenced by immunoelectrophoretic and quantitation studies.

7. The IgM concentration in bile of bursectomised ducklings was lower than that in the control group.

8. Egg yolk of ducks contained both IgA and IgG.

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IMMUNOGLOBULINS IN DUCKS AND ROLE OF BURSA OF FABRICIUS IN THEIR PRODUCTION

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ABSTRACT OF A THESIS

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ABSTRACT

A study was undertaken to determine the immunoglobulin profile of ducks and to delineate the role of bursa in their production.

Among the four different methods of bursectomy employed in the study, Cy was found to produce the maximum reduction in body weight compared to other treatments. Marked reduction in bursal weight was also produced by Cy compared to T and ABS groups, while in SBx group the bursa was absent *in toto*. Surgical bursectomy resulted in significant reduction in spleen size of both surgically bursectomised uninoculated and inoculated ducklings.

Histopathological studies revealed that the bursal development was highly suppressed on treatment with Cy. The ABS and testosterone treatments also elicited suppressive effect on bursa, but to a comparatively milder extent. It was evident that bursa had a role in lymphoproliferative reactions of spleen, as indicated by the maximum suppressive effect on spleen by SBx group.

Ammonium sulphate at 33 % level was found to be ideal for fractionation of duck serum globulins.

Two main elution peaks were obtained on subjecting ammonium sulphate precipitated globulins to sephadex G-200 chromatography. Concentrated and rerun ascending fractions of first major peak yielded purified IgM while those of the second major peak yielded purified IgG.

Comparing the three age groups of antigen inoculation in ducklings bursectomised by different methods, total protein levels lower than the control were observed only in SBxSR (groups I and II) and SBxSt (groups I and III).

Bacterial agglutination test revealed that in all four groups of bursectomised ducklings, antibody titres far below those of controls were produced.

In SRBC agglutination test, lowered antibody titres than the control were observed in groups I to III of SBx, Cy and T-treated and groups II and III of ABS treated birds. Group I ABS administered ducklings had identical titres as that of control at days 7-21 post-inoculation.

Bursectomised uninoculated ducklings revealed higher IgM levels than age-matched controls at many of the weeks under study. In bursectomised ducklings administered SRBC, IgM values higher than control level were obtained in the following cases: in groups I and II of SBxSR and TSR; groups II and III of CySR; and groups I to III of ABSR. S. typhimurium inoculated bursectomised ducklings revealed in the following treatments, higher IgM levels than control; in SBxSt groups I and II; CySt groups I to III; TSt group I; and in ABSt groups II and III.

Quantitation of IgG levels in bursectomised ducklings revealed lower than control levels in SBx, Cy and T groups at 1-4 weeks of age, while the levels were higher or lower

or identical with that of control from week 5. In ABS group the level was lower at 1-2 weeks.

In bursectomised ducklings administered SRBC, higher IgG levels than control were obtained in groups II and III of SBxSR, CySR, TSR and ABSR. In group I, treated ducklings revealed either lower or identical IgG levels compared to age matched controls. S. typhimurium inoculated bursectomised ducklings had higher IgG levels compared to CSt in all three groups of inoculation.

Bile of ducks was found to contain only IgM, as evidenced by immunoelectrophoretic and quantitation studies. The IgM level in bile of bursectomised ducklings was found to be lower than that of the control.

Yolk of duck eggs contained both IgM and IgG.

Significantly higher lymphocyte count between the control and treated groups under study was detected at 4th (in SBx and T) and 8th weeks (in Cy). At 7th week, SBx group had significantly lower lymphocyte count, compared to control.

At the fourth week of age, SBx and T groups had significantly lowered heterophil counts, compared to control. SBx group showed significantly higher count than the control at the 7th week, while at the 8th week, Cy-treated birds had markedly lower count, compared to the control.

Eosinophil counts in bursectomised ducklings were higher than in control, while the basophil and monocyte counts in control and treated groups were more or less the same.

The results obtained from the present study revealed that,

1. Among the different methods of Bx employed Cy produced maximum reduction in body weight, while SBx resulted in total elimination of bursa and significant reduction of spleen size.
2. Bursa had a role in lymphoproliferative reactions of spleen. Cy- produced maximum suppressive effect in bursa while in spleen SBx caused maximum suppression.
3. Ammonium sulphate (33%) was ideal for separation of duck serum globulins.
4. Sephadex G-200 gel filtration was suitable for purification of IgM and IgG of ducks.
5. Bursa was concerned only with specific antibody production.
6. Elevated IgM and IgG levels were produced in bursectomised birds by extra-bursal B cells.
7. Bile of ducks contained IgM, the concentration of which was lower than the control in bursectomised birds.
8. Egg yolk of ducks contained both IgM and IgG.