Short title

IMMUNOSUPPRESSION AND ITS EFFECT ON AVIAN COCCIDIOSIS

Araujo

ABSTRACT

Ph.D. HYMA RITA B. ARAUJO Parasitology

STUDIES ON IMMUNOSUPPRESSION IN THE CHICKEN AND ITS EFFECT ON *EIMERIA TENELLA* INFECTION

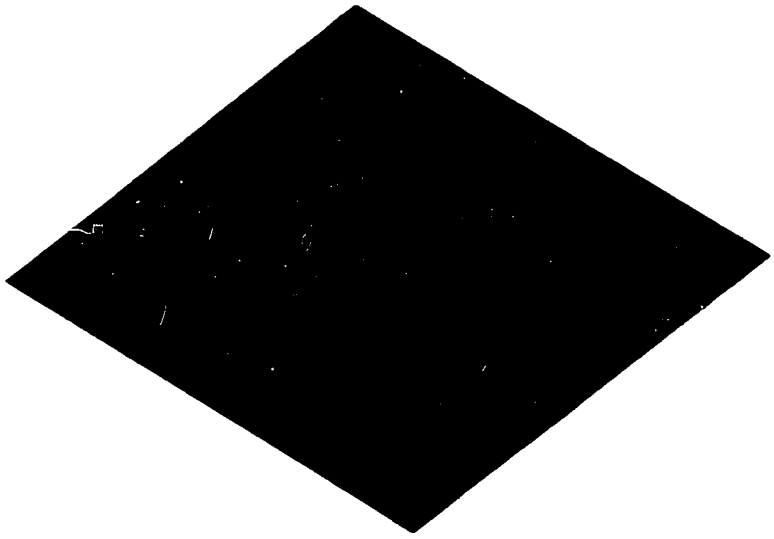
The effects of several immunosuppressive treatments on hemagglutinin synthesis, skin homograft reaction and *Eimeria tenella* infection in the domestic fowl were investigated. The present study confirmed the complex nature of the inter-relationship between the integrity of the immunological state of the host and the regulation of parasite numbers. Immunological competence to sheep erythrocytes was seriously impaired by hormonal bursectomy (HBx), gamma irradiation at a dose of 800 rads at three weeks of age (800R/3 wk), neonatal thymectomy in conjunction with 700 rads gamma irradiation at three weeks of age (NTx + 700R/3 wk), and by antithymus globulin (ATG) and antibursa globulin (ABG) treatments, but a possible suppression of host resistance to *E. tenella* infection was indicated only in the latter three groups by an increase in the total number of oöcysts produced per chicken. Treatment with NTx + 700R/3 wk also suppressed the homograft response; but oöcyst production per bird was relatively higher in the ABG-treated group where impairment of the immunological response was not so great.

ABREGE

Ph.D. HYMA RITA B. ARAUJO Parasitologie

ETUDE DE L'IMMUNO-SUPPRESSION CHEZ LE POULET ET SES EFFETS SUR L'INFECTION A EIMERIA TENELLA

Les effets de divers traitements immuno-suppressifs sur la synthèse des hémagglutinines, la réaction homogreffe de peau, et l'infection à Eimeria tenella furent étudiées chez le poulet. Cette étude confirme la nature complexe des relations éxistant entre le système immunologique de l'hôte et le contrôle du nombre de parasites. La production d'anticorps vis-à-vis les globules rouges de mouton fût sérieusement touchée lorsque les poulets étaient traités par la boursectomie hormonale (HBx), irradiation à 800 rads des rayons gamma à l'âge de trois semaines (800R/3 wk), thymectomie néonatale en plus de l'irradiation à 700 rads des rayons gamma à l'âge de trois semaines (NTx + 700R/3 wk) et finalement, traitement des poulets avec la globuline anti-thymus ou anti-bourse. D'après les résultats obtenus, la résistance de l'hôte à E. tenella semble affectée seulement dans les trois derniers traitements, où l'on a noté une augmentation du nombre d'oöcystes produits par poulet. Le traitement NTx + 700R/3 wk supprime la réaction homogreffe et augmente la production d'oöcystes, mais cette augmentation est cependant inférieure que chez le groupe de poulets traité à la globuline anti-bourse. Ce dernier traitement n'a pas affecté le système immunologique aussi profondément que NTx + 700R/3 wk.



STUDIES ON IMMUNOSUPPRESSION IN THE CHICKEN AND ITS EFFECT ON *EIMERIA TENELLA* INFECTION

by

Hyma Rita B. Araujo

A thesis presented to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Institute of Parasitology
McGill University
Montreal

October 1971

ACKNOWLEDGEMENTS

The author wishes to express her deep gratitude to Dr. Charles E. Tanner, Associate Professor at the Institute of Parasitology, McGill University, under whose direction this investigation was carried out, for his valuable suggestions and criticism throughout the study. Thanks are also due to Dr. Tanner for undertaking the tedious task of fractionating the various types of antilymphocyte gamma globulins by DEAE-column chromatography.

The writer is also indebted to:

Dr. Robert O. Hawes of the Animal Science Department of Macdonald College and his staff, for continuously providing fertile eggs and for the privilege of using the incubators and other facilities.

Dr. Edmund S. Idziak of the Microbiology Department, for his valuable assistance in the use of the Gamma cell and for supplying the attenuators.

Drs. David Campbell and Bruce Downey of the Ayerst Laboratories, Ville St. Laurent, P.Q., for providing cultures of sporulated oöcysts of Eimeria spp.

Professor K. G. Davey, Dr. Manfred Rau and Dr. Erwin Huebner of the Institute of Parasitology, for their help with photography.

Dr. Nicholas Blitz, for his assistance in translating German papers.

Mr. Gaétan Faubert, for translating the abstract into French.

Mr. John Rogerson, for his patient and critical reading of the manuscript.

Special thanks are extended to each and every member of the Institute of Parasitology, without whose continued interest this work could not have progressed well.

The financial assistance from the Agricultural Research Council of the Province of Quebec, in the form of a grant (No. McA-68-321), is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

Figure Page

I. INTRODUCTION

Coccidiosis is an infection of the digestive tract, caused by protozoan parasites belonging to the class Sporozoa. Although it is well established that infection by these parasites produces acquired immunity (Beach and Corl, 1925; Tyzzer, 1929), the exact mechanisms involved in host resistance are very little understood. Experimental evidence so far obtained has failed to implicate a significant role for humoral antibody; most investigators believe that resistance to reinfection involves cellular factors.

Unlike mammals, birds demonstrate some degree of dissociation of the cellular and humoral immune mechanisms: the circulating antibody production is chiefly under the "control" of the bursa of Fabricius, and the thymus is the principal source of those cells which mediate immunological reactions associated with delayed hypersensitivity. Both the thymus and the bursa act as "central lymphoid organs" and supply lymphoid elements to the spleen, intestine and other "peripheral lymphoid tissues."

A study of the immunity to Eimeria infections in birds is complicated by the fact that very little is

understood about the nature of the fowl antibody and the morphology and functions of the cells associated with it. Furthermore, isolation of the different stages of the parasite for the preparation of specific antigens is very difficult since the complete separation of the parasite from host tissue would be nearly impossible. Recent techniques used for studying the immune mechanisms in coccidiosis have involved the suppression of immunity by ablation of lymphoid organs, irradiation and treatment with cortisone or antilymphocyte serum. For example, Long and Pierce (1963), and Pierce and Long (1965) have indicated that acquired immunity against coccidiosis is dependent on thymus-controlled cellular factors. The purpose of the present study was to clarify the nature of the immunological control of coccidiosis thoroughly with the help of effective immunosuppressive techniques. Many methods for the suppression of the immune response were reinvestigated in similar experiments to determine the most effective of these techniques. The results of this preliminary investigation were then applied to a study of host resistance against Eimeria tenella infections in White Leghorn chickens.

II. REVIEW OF THE LITERATURE

A. Coccidiosis

(i) General

Coccidiosis affects a wide variety of animals including cattle, sheep, goats, rabbits and both wild and domesticated birds. This disease is economically important since it accounts for about ten per cent of the annual mortality of chickens in North America (Davies et al., 1963).

The nature of this disease was little understood before the detailed studies of Tyzzer and his co-workers (1929, 1932) which indicated the adverse effect of the disease on weight gains, feed consumption and egg production; chronic infection with these parasites damages the lining of the intestinal tract, thus reducing the efficiency of digestion and absorption. Infected birds become weak and can fall victim to other diseases. Coccidiosis has been kept under control by the use of chemotherapeutic agents such as sulfaguanine, sulfaquinoxaline and Nicarbazin. In recent years, however, it has become evident that some of these parasites can develop a considerable degree of resistance to these drugs, complicating the problems of poultry management.

Eight species of Eimeria are responsible for chicken coccidiosis; they are monoxenous parasites, characterized by an extraordinary degree of host specificity and are strictly confined to specific regions of the small intestine, rectum, caeca or other parts of the digestive tract. The commonest and the most pathogenic species of coccidia is the widely distributed Eimeria tenella (Railliet and Lucet, 1891) Fantham 1909. This species chiefly affects young chickens up to the age of eight weeks, with a peak incidence at five weeks of age. Infection is normally confined to the caecum but may occasionally involve parts of the small and large intestines adjacent to the caecal junction (Tyzzer, 1929).

Infection is produced after the ingestion of sporulated oöcysts which excyst in the intestine; pancreatic juice is believed to help this process (Levine, 1942c; Ikeda, 1956a, 1956b). Sporozoites released from the oöcysts penetrate the basement membrane and the tunica propria and finally gain access to the epithelial cells lining the glandular crypts. Between 24 and 48 hours after infection, the sporozoites develop intracellularly into first-generation schizonts measuring approximately 24 μ x 17 μ, each containing about 900 merozoites (Tyzzer, 1929). These first-generation merozoites are very small, measuring about 3 μ in length and are characterized by a terminal

granule at the pointed end. After release from the schizonts into the lumen of the caecum, the merozoites invade other epithelial cells which round off, increase in size and migrate into the subepithelial tissue layers. Within 72 hours of the initial infection these merozoites mature into second-generation schizonts which measure up to 50 μ in diameter. These new schizonts soon contain numerous 16 μ long, second-generation merozoites which also possess a terminal granule at the pointed end; the latter are eventually released into the caecal lumen.

The second generation merozoites penetrate new caecal epithelial cells but now develop into gamonts which in turn produce micro- and macrogametes. Syngamy results in zygotes which mature to form oöcysts with an average size of 23.0 μ x 19.2 μ; these begin to appear in the faeces seven days after infection, their numbers rise to a peak by the tenth day and then rapidly decrease. According to Herrick (1936), under exceptional circumstances the oöcysts may persist in the caecum for as long as seven and a half months. The oöcysts undergo sporulation and become infective 48 hours after having been passed out in the faeces. Under average laboratory conditions, sporulated oöcysts will remain infective for about three weeks, when stored in two per cent potassium dichromate solution; they can be killed within 24 hours at a temperature of 102.2°F and a relative humidity of 47 per cent (Ellis, 1938).

A heavy infection with E. tenella is characterized by a frequently lethal, copious haemorrhage on the fifth and sixth days of infection due to the massive destruction of caecal epithelium by the large second generation schizonts. The caecal wall of the infected birds appears whitish because of the accumulation of oöcysts in the caecal glands. The epithelial lining is degenerated and the caecal lumen becomes filled with a core of necrotic tissue consisting of decomposing tissue cells, blood and developmental stages of the parasite - particularly oöcysts (Davies et al., 1963); this caecal core is usually expelled within 14 days.

(ii) Immunity

It was recognized very early that animals which recover from an infection with Eimeria are frequently resistant to reinfection. Coccidiosis is typically a disease of young stock and the resistance of older birds is due to specifically acquired immunity (Horton-Smith, 1947b). Tyzzer (1929) was the first to recognize that resistance to coccidia was specific so that infection with any one species did not provide resistance to another. These parasites are also highly host-specific: for example, turkeys, geese and pheasants are not susceptible to infection by Eimeria tenella of the chicken (Leathem, 1966).

Furthermore, there is evidence (Rosenberg, 1941; Rosenberg et al., 1948; Long, 1968) which suggests that some strains of birds possess a higher innate resistance than others.

Tyzzer (1929) indicated that higher levels of protection are induced by those species of Eimeria which penetrate deeply and tend to be retained in the host tissues. The size of the immunizing dose also plays a role in the establishment of the immune state; severe infections with E. tenella were shown (Tyzzer, 1929) to elicit a well-marked and prompt immunity, whereas slight infections with only a few oöcysts did not protect against massive reinfections. Waletzkey and Hughes (1949) found, however, that a single severe infection does not always confer a "solid" immunity to the development of caecal lesions, although it prevents mortality from reinfection. Pierce et al. (1962) demonstrated that three graded doses of oöcysts were sufficient to produce complete immunity to E. tenella infection. These fowls were found, however, to be capable of supporting parasites in sufficient numbers to produce a few oöcysts in their faeces. Protection need not necessarily have a strict immunological basis since reinfection can be prevented by pathological changes of the intestinal tract such as caecal "cores" and a thickening of the caecal walls; these changes may persist for three or more weeks (Waletzky and Hughes, 1949). It is

interesting to note that, although the duration of complete immunity lasts for only 42 to 63 days, the host is protected against fatal E. tenella infection for about 105 days following immunization (Leathem, 1966).

There are conflicting reports regarding the type of immune mechanisms involved in coccidial infection. Several workers (McDermott and Stauber, 1954; Itagaki and Tsubokura, 1955; Burns, 1958; Horton-Smith et al., 1961; Rose and Long, 1962; Pierce et al., 1962; Long et al., 1963; Herlich, 1965) demonstrated humoral factors such as lysins, precipitins and agglutinins in the sera of infected chickens; agglutinating antibodies have also been detected in the immune sera of calves infected with Eimeria bovis (Anderson et al., 1965). Similarly, Rose (1959a, 1961) demonstrated precipitins in rabbit sera against E. stiedae infection; the maximum number of precipitation bands was given by sera withdrawn at the time when the largest number of developmental stages coexisted in the host. Immune serum was also shown to be capable of lysing sporozoites and merozoites in vitro (Long et al., 1963; Herlich, 1965; Anderson et al., 1965). Some investigators (Pierce et al., 1963; Fitzgerald, 1964; Pierce and Long, 1965; Cerna, 1966) have concluded, however, that these humoral antibodies may not play a significant role in the mechanisms involved in host resistance. On the other hand, Rose (1971) has

recently been able to protect chickens against E. maxima infection by the passive transfer of sera from infected birds.

Some of the antibodies produced in coccidial infections may be analogous to those involved in other mucosal infections of bacterial, viral and protozoan origins where precipitating or agglutinating antibodies not observed in the serum have been identified in the excreta of the host (Augustin and Ridges, 1963). These faecal antibodies have been termed muco- or copro-antibodies by Pierce (1959). They are present in the mucin at the surface of mucous membranes and are believed to be derived either from sites in the immediate vicinity of the infection or extravasated from the plasma. Some of these antibodies in the fowl may also be analogous to the IgA class of immunoglobulins whose presence has been confirmed in secretions of mammals (Tomasi and Bienenstock, 1968). Copro-antibodies may possess some protective properties, probably in the form of local resistance to infection by pathogenic organisms. These antibodies are likely to have the greatest effect on pathogens which are neither invasive nor liberate toxic products, but which are confined to the mucous surface or to the superficial layers of the mucous membrane.

Despite the belief by some that circulating antibodies may have very little direct significance in intestinal infections, they may, however, diffuse into the site of infection in sufficient concentration to react against the pathogenic organism (Pierce, 1959); such antiparasitic factors could affect Eimeria sporozoites in the lumen or on the mucous surfaces and even within host cells (Rose and Long, 1969). Support for an intracellular action of antibodies can be found in the work of Hammond et al. (1964), who reported that the penetration of host cells by first-generation merozoites of E. bovis was inhibited in immunized calves.

Coccidial infection has been shown to be accompanied by a local infiltration of granular leucocytes (Clarkson, 1958, 1959a). These cells increase in number, especially on the fourth and fifth days when second-generation schizonts are developing and maturing (Pierce et al., 1962). According to Long and Pierce (1963), some of the pyroninophilic cells infiltrating into the submucosa closely resemble the globule leucocytes or Schollenleukozyten of Weil (1920) and Mjassojedoff (1926) which have been associated with antibody formation (Dobson, 1966). Such cellular response in the intestine of infected chickens may have an important role in the control of coccidiosis. Rommel and Heydorn (1971) recently succeeded in adoptively

transferring to normal rats, an immunity to Eimeria nieschulzi by the intraperitoneal injection of lymphocytes from infected animals.

B. The lymphoid organs and their role in immune reactions

The lymphoid tissues of the chicken consist chiefly of the thymus, the bursa of Fabricius, the spleen, the tonsilla caecalis and the Peyer's patch-type of cell accumulations in the walls of the gastro-intestinal tract (Janković, 1968). Unencapsulated lymph nodules and "ectopic" lymphoid areas are found in practically all organs; they are not derived from pre-existing clusters of lymphoid cells but seem to arise de novo from lymphocytes in response to local infection or irritation (Biggs, 1956).

The thymus is a paired gland, located subcutaneously on either side of the neck. It is formed of several flattened oval masses of tissue along the course of the jugular vein. The mature thymus contains areas of lymphoid cell accumulations called lymphoid follicles which are divided into cortical and medullary tissues; large eosinophils, lymphocytes, reticular cells and special structures called Hassal's corpuscles, which are spherical bodies composed of concentrically arranged cells, are present in the medulla. Although the normal mammalian thymus contains

few plasma cells and no germinal centers, both have been observed in 12-week-old chickens (Thorbecke et al., 1957). In the ring-necked pheasant this organ reaches its maximum size at 100 or 130 days of age, coinciding with the stage of most rapid body growth. It becomes completely involuted before sexual maturation, and is believed to be controlled by adrenal cortical and sex hormones (Hublé, 1958). Warner (1964) showed that in the chicken, only the thymic cortex involutes and that the medullary cells remain immunologically competent and persist throughout the life of the animal.

The bursa of Fabricius is a sac-like organ situated dorsal to the cloaca, with its lumen in direct contact with the latter. The bursa is surrounded by a thin layer of circular muscle beneath which is a vascularized connective tissue zone; the lumen is lined with columnar epithelial cells, characterized by longitudinal folds which contain numerous lymph follicles. As in the thymus, these follicles are also divided into cortical and medullary regions. The medulla in many lobules seems to be free from lymphocytes and presents only a reticular framework (Malewitz and Calhoun, 1958). The weight of the bursa varies in different strains of chickens, but it usually reaches a size of 2 to 3 cm x 1.5 cm, four to five months after hatching and disappears by ten months of age. The time of involution of this gland

varies in different breeds of chickens; involution is believed to be under the control of adrenal cortical (Kirkpatrick, 1944) and sex hormones (Auerbach, 1962).

The spleen is an oval, reddish brown organ, situated immediately to the right of the junction of the glandular and muscular stomachs. It is enclosed by a thin serous membrane and is traversed by septa known as trabeculae. The spleen is believed to originate from the mesoderm. Unlike the thymus and the bursa, the lymphoid nodules (follicles) in the avian spleen do not involute either before or during sexual maturation.

Two distinctly different types of lymphoid tissues are present in the mature spleen. One is seen along the small arteries and arterioles as sheaths of small lymphocytes or as scattered clusters composed of large and small lymphocytes, lymphoblasts and primitive reticular cells containing frequent mitotic figures. This represents the red pulp and forms the bulk of the lymphoid tissue in this organ; it becomes apparent in the normal chick during the immediate post-hatching period (Cooper et al., 1965). This tissue is believed to develop under the influence of the thymus. A second type of lymphoid tissue, usually termed the white pulp, forms sharply circumscribed round or oval lymphoid follicles, apparently encased in a thin fibrous membrane; it always lies in juxtaposition to a small artery.

The development of these follicles as well as plasmacytic cells within the spleen is believed to be under the control of the bursa of Fabricius. The white pulp develops late in the normal chicken, at about the fourth to fifth week of life (Cooper et al., 1965).

The wall of the digestive tract also contains sufficient accumulations of lymphoid cells to make it one of the major subdivisions of the lymphoid system (Janković and Mitrović, 1967). Otte (1928) has described areas in the digestive tract of the chicken having the appearance of Peyer's patches; these "nodules" were found most abundantly in the duodenum. Looper and Looper (1929) reported that lymph nodules associated with the tunica propria and submucosa of the blind ends of the caeca make their appearance about 14 days after hatching. According to these authors the caecal mucosa is later obliterated by lymph nodules and the circular layers of muscle in the blind end become displaced by lymphoid tissue. The tonsilla caecalis consists of diffuse masses of lymphoid cells and many germinal centers situated near the proximal end of the caeca. The cellular make-up of the tonsillar germinal center closely resembles that of the germinal center of the spleen (Janković, 1968).

The lymphoid organs begin to differentiate during the early period of embryonic development. On the seventh

embryonic day, "undifferentiated" epithelial cells transform in the thymus, either into lymphoblasts or into stellate reticular epithelial cells. Maturation of the lymphocytes is completed by the tenth or eleventh day and the thymus then assumes its predominantly lymphoid character (Ackerman and Knouff, 1964). The bursal primordium appears in the chick on the fifth embryonic day (Meyer et al., 1959). By the seventh day it forms a tubular structure and its terminal end enlarges into a vesicle on the tenth day. Ackerman and Knouff (1959) and Meyer et al. (1959) have reported that lymphoid nodules first appear on the 12th day as purely endodermal proliferations. Lymphocytic proliferation in the bursa occurs by the 12th to 14th day of incubation. The spleen makes its appearance when the embryo is nearly four days old (Danchakoff, 1916). The differentiation of small lymphocytes in the spleen is preceded by the development of generations of large lymphocytes (hemocytoblasts). The first groups of small lymphocytes differentiate between the 15th and 17th days of embryonic development and a number of mesenchymal and endothelial cells are gradually transformed into typical macrophages (Evans, 1915). The spleen and intestine lack demonstrable lymphoid follicles at the time of hatching; they develop in these secondary sites at one to two weeks of age.

Lymphocytes accumulate and reproduce in nodules and loose aggregations, in the intestine, in the white pulp of the spleen and in the cortex of the lymph nodes. In response to antigenic stimulation lymphocytes differentiate into plasma cells which are capable of antibody synthesis. The latter concentrate in the lamina propria of the intestine, in the red pulp of the spleen and in the medullary cords of the lymph nodes. In most lymphoid organs lymphocytes proliferate in areas called germinal centers. These centers are absent from the thymus even though this organ is the chief site of lymphopoiesis (Clark, 1963). Germinal centers and plasma cells do not become prominent features of the various lymphoid tissues until exposure of the animal to antigens after birth (Bridges et al., 1959).

The thymus is the major site of lymphopoiesis, at least in young animals, and the spleen and other lymphoid organs are considered to be secondary sites where lymphocytes from the primary lymphoid organs accumulate and where proliferation is of little importance in the maintenance of the cell population. It has been suggested (Ruth, 1960) that some of the Foá-Kurloff cells (F cells) in the spleen originate in the thymus and that these cells provide what may be the first cytological proof of a selective transfer of thymic cells to the spleen. The

thymus also produces a lymphocytosis-stimulating factor (Metcalf, 1958). In spite of the effect of this factor, the greatest single force in lymphocyte production appears to be the inherent proliferative activity of the tissue itself. According to Miller (1962), Warner and Szenberg (1962, 1964), and Aspinall et al. (1963), the thymus effects the development of cells concerned with cell-mediated immune responses such as graft-versus-host reactions, delayed hypersensitivity and most homograft responses.

In mammals, interaction between cells derived from the bone marrow and thymus is believed to be required for immune reactions (Claman et al., 1966; Davies et al., 1966, 1967; Mitchell and Miller, 1968; Nossal et al., 1968). In tuberculin hypersensitivity the thymus is required for active initial sensitization but the overwhelming majority of cells which leave the circulation and infiltrate the skin reaction sites are non-specific marrow-derived monocytes (Lubaroff and Waksman, 1968; Lidén, 1967). Gowans et al. (1962), using tracer techniques in rats, have indicated that thymus-derived small lymphocytes probably initiate the reaction against first-set skin homografts. Antibody-like reactive sites are created in or at the surface of sensitive lymphoid cells as a result of specific and selective adsorption of antigen(s) responsible

for homograft sensitivity (Berrian and Brent, 1958). In rats some of the small lymphocytes in the regional lymph nodes draining skin transplants react with graft antigens and give rise to large pyroninophilic cells (Gowans et al., 1962). These pyroninophilic cells in turn differentiate into a new group of small lymphocytes which leaves the nodes, enters the blood stream and invades the graft.

The mechanism by which immunologically competent cells destroy the cells of the homograft remains in doubt. The majority opinion holds that death of the graft results from the interaction of antibodies closely associated with these cells and the transplantation antigens in the donor tissue (Wiener et al., 1964). This mechanism may be mediated by a cell-bound immune factor or factors. This factor is not considered to be an antibody of the conventional type; it resists freezing and thawing, water lysis and enzymatic treatments (Medawar, 1946). It can be stored for several months and upon incubation, is readily liberated from leucocytes into the cell-free supernatant (Lawrence, 1959).

The lysis of donor tissue cells is believed to be effected by complement; when the cells lyse, intensely proteolytic enzymes are released from lysosomes and condiderable further damage and disintegration are produced. This reaction may be accelerated or hindered by the

presence of humoral antibodies (Amos, 1962). Humoral antibody production associated with the homograft reaction has been indicated by several workers. Iwasaki et al. (1967) demonstrated an IgG antibody in the blood of patients following kidney transplantation which fixed specifically to donor kidney cells; this antibody could be detected as early as two days after grafting. In rats, cytotoxic and hemagglutinating antibodies have been detected seven days after renal homotransplantation (Guttman et al., 1967). IgG and IgM complement-fixing antibodies bound to the donor kidney have been eluted from rejecting canine renal homografts (Hampers et al., 1967). Nonetheless, in all of these reports, a strong causal relationship of antibody to rejection has been lacking (Spong et al., 1968).

According to Lawrence (1959), the mechanism of tissue damage in graft rejection is analogous to that of tuberculin sensitivity. The case for regarding transplantation immunity and hypersensitivity reaction as different manifestations of the same immunological response is supported by the fact that both are induced by the transplantation of skin homografts or by the injection of allogeneic cells. Furthermore, both reactions arise and decay at the same rate and both can be transferred by sensitized lymphoid cells but not by serum (Brent et al., 1962).

The production of circulating antibody is directly associated with the spleen. In mammals, the cells which synthesize and release the antibody are probably derived from the bone marrow (Nossal et al., 1968), whereas in the chicken, antibody synthesis is chiefly controlled by the bursa of Fabricius (St. Pierre and Ackerman, 1966). It has been shown that cells migrate from the bursa to the spleen and other secondary lymphoid tissues (Woods and Linna, 1965). Evidence (Glick, 1958; Janković and Leskovič, 1965; St. Pierre and Ackerman, 1965) strongly indicates that the bursa may also elaborate a humoral factor capable of inducing immunological reactivity in unstimulated lymphoid cells. The differentiation of plasma cells, however, may not depend entirely on this factor alone.

In the chick embryo, gamma globulin synthesis starts by the 11th day of incubation (Ebert and DeLanney, 1960). Nonetheless, the synthesis of most immunoglobulin is believed to take place only in response to antigenic stimulation during the postnatal period. Wolfe and Dilks (1948) showed that newly hatched chicks could be stimulated to produce a low titer of precipitins against bovine serum. A gradual increase in this ability was noted up to four weeks, with "serological maturity" being attained by about five weeks of age; no increase in titer occurred thereafter.

As in cellular immune reactions, an early and characteristic feature of the humoral response to antigen in mammals is the appearance of large pyroninophilic cells throughout the spleen. These cells have been shown to originate in the white pulp, and subsequently migrate to the red pulp (Congdon and Makinodan, 1961; Thorbecke et al., 1962; Langevoort, 1963) where plasma cells first appear (Fagraeus, 1948). Hanna et al. (1966) have indicated that the fine structural changes in the pyroninophilic cells are characteristic of active protein synthesis and that these are the cells which differentiate into plasma cells. In the chicken spleen, antibody-containing cells with the morphology of haemocytoblast and immature plasma cells appear in the red pulp by the second day after injection of human serum albumin; antigen is also found in the red pulp strands at the same time (White, 1963). The percentage of plasmacytic cells was shown to increase continuously for 72 hours after the intravenous injection of sheep red blood cells; this proliferation was correlated with the release of a significant amount of antibody by spleen cells (Abramoff and Brien, 1968). Similar morphologic changes were also noticed by these authors in the bursa of Fabricius; this might be associated with a secretory function which may influence cellular differentiation in the spleen.

Černý and Iványi (1966) found that during the peak production of antibody-containing cells in mammals, antibodies in the serum were of the IgM type and the appearance of IgG antibodies coincided with the differentiation of plasma cells. According to Playfair et al. (1965), each germinating area in the spleen is formed of a single precursor lymphocyte, restricted to forming a single antibody. These authors demonstrated that, in the mouse, cells forming specific antibodies to one antigen became non-randomly distributed throughout the spleen. If the animal was immunized with two different antigens, the distribution of spleen cells forming antibody to one antigen differed significantly from that of cells producing antibody to the other.

Fowl antibodies differ in a number of respects from those typically formed by mammals. With mammalian antibodies, a decrease in in vitro precipitation occurs when the NaCl concentration is increased above one per cent. Maximal precipitation of chicken antibodies is obtained in salt concentration much greater than this: a NaCl concentration of eight per cent is necessary to obtain an accurate quantitative assay of the antibodies in fowl antiserum (Goodman et al., 1951). This phenomenon was defined by Benedict et al. (1963), who presented evidence indicating that primary chicken antiserum contains a high

molecular weight antibody and a 7S antibody, which gives precipitates in 0.15 M NaCl and which agglutinates sensitized red cells; this latter immunoglubulin is found in high concentrations in hyperimmune sera. A 7S non-hemagglutinating antibody was also found which precipitates with antigen only in 1.5 M NaCl, when it is isolated from the heavier fraction by zone ultra-centrifugation. Experiments conducted by Orlans et al. (1961) revealed that fowl antiserum to rabbit globulin contained two types of homologous antibodies having the same electrophoretic mobility but giving two distinct bands of precipitation. One type of antibody had a molecular weight of about 600,000 and the other 180,000. The latter type formed precipitates in 0.9 and 8 per cent NaCl solutions, but larger precipitates formed at the higher salt concentration contained a component that was neither antigen nor antibody. Deutsch et al. (1949) showed that unlike mammalian antisera, alpha globulin contributed to the precipitating power of the gamma globulin in chicken antisera. It has also been indicated (Wolfe, 1942; Wolfe and Dilks, 1946) that the in vitro interfacial "ring test" titer of fowl antiserum rises markedly during storage, to a maximum by about 12 days after bleeding.

C. Immunosuppression

(i) Introduction

Immunological unresponsiveness (tolerance) is a condition in which an animal will not respond to an antigenic stimulus; such a state can be induced by the removal of lymphoid organs, treatment with sublethal doses of radiation, or by the administration of antilymphocyte serum, corticosteroids, or certain chemical compounds which block antibody production at the metabolic level. Tolerance can also be induced by the injection of massive doses of antigen into the adult animal, of low doses during the neonatal period, and by the transfusion of specific antisera.

The beginnings of the current concept of immunological tolerance date from the observations of Owen (1945) on chimaerism in twin cattle. Such twins accept skin grafts from each other, but not from a third party. These observations led Burnet and Fenner (1949) to propose that the recognition of "self" is not genetically determined, but that it is acquired during ontogenesis when immunological competence to foreign antigens develops in the presence of auto-antigens; immunological responsiveness to the latter is suppressed during development.

A persistent blood chimaerism is induced after embryonic parabiosis in chickens. Similar to Owen's cattle twins, these chickens tolerate grafts exchanged between them (Billingham et al., 1956); they are also incapable of producing agglutinins against the erythrocytes of the partner (Hašek and Hraba, 1955). Complete suppression of antibody formation and skin homograft rejection was also obtained after the injection of donor erythrocytes or leucocytes into embryos of normally fully competent recipient chickens (Hašek, 1956). Tempelis et al. (1958) obtained an almost complete inhibition of immunological reaction to bovine serum albumin in normally responsive fowls after 15-day-old embryos were injected with a heavy dose of this antigen; the tolerance, however, disappeared by twelve weeks of age. According to Simonsen (1956) and Hirata and Schechtman (1960), the most susceptible stage of the chick embryo for the induction of immunological tolerance is between the 15th and the 17th day of incubation.

Immunological tolerance can be induced in chicks even after hatching. Bellingham et al. (1956) stated that a newborn chick is at the very end of the epoch of life during which it can respond to an antigenic stimulus by becoming tolerant. These authors showed that a marked suppression in the immune response to donor skin homografts

by adult chickens could be obtained after the injection of donor whole blood into newly hatched chicks. Mitchison (1962) was able to induce some tolerance to foreign erythrocytes by transferring irradiated whole blood into 8-day-old chicks. Wolfe et al. (1957) also found that injection of massive doses of bovine serum albumin at hatching results in temporary immunological tolerance in the fowl.

(ii) Ablation of the lymphoid organs

It has been known for a long time that surgical removal of the thymus gland in mammals in the early neonatal period affects the cellular immune processes and heavily depletes the pool of small lymphocytes in the circulation and in the tissues (Good and Gabrielsen, 1964). Neonatal thymectomy in chickens also produces a similar depletion in the peripheral and tissue lymphocyte population, including the bursa of Fabricius (Janković and Isaković, 1964; Yamaguchi et al., 1964) without affecting plasma cell proliferation in the spleen (Isaković et al., 1963).

Warner et al. (1962), Warner and Szenberg (1962) and Aspinall et al. (1963) found that removal of 90-95 per cent of the neonatal thymus of chickens considerably prolonged the rejection time of skin homografts; normal

rejection did not occur until the residual thymic tissue could regenerate and produce sufficient numbers of appropriate immunologically competent cells. Inhibition of thymic development by the prenatal or neonatal injection of corticosteroids into the chick is also effective in delaying the rejection of homografts (Aspinall and Meyer, 1964). Neonatal thymectomy suppresses the ability of chickens to produce experimental allergic encephalomeylitis (Janković and Išvaneski, 1963) and experimental allergic thyroiditis (Janković et al., 1965). Pierce and Long (1965) showed that removal of the thymus during the neonatal period could partially impair the immune response against Eimeria tenella.

Ablation of the bursa of Fabricius early in life can greatly curtail the capacity of the maturing animal to produce circulating antibodies (Glick et al., 1956; Mueller et al., 1960; Papermaster et al., 1962; Isaković et al., 1963; Long and Pierce, 1963; Ortega and Der, 1964; Carey and Warner, 1964) and inhibit lymphoid development in the spleen and in the intestine (Danchakoff, 1916). The studies of Isaković and Janković (1964) have shown that plasma cell proliferation does not take place in neonatally bursectomized chickens, in response to an antigenic stimulus. Neonatal bursectomy, when supplemented by cauterization of the bursal duct lining, causes a profound

depression in the level of 7S gamma globulin but not of β2-macroglobulin or any other serum protein (Ortega and Der, 1964). Gamma globulin levels did not return to normal during the seven months that these bursectomized and cauterized birds were observed. When surgical removal of the bursa is followed by treatment with heavy doses of radiation, an absolute inhibition of circulating antibody synthesis can occur despite the presence of germinal lymphoid centers and plasma cells in the splenic and intestinal tissues (Cooper et al., 1966). Although neonatal bursectomy does not generally have a significant effect on cellular immune mechanisms (Aspinall et al., 1963; Yamaguchi et al., 1964), Ruth et al. (1964) demonstrated that the bursa of Fabricius could play a role in the immune response to such transplantation antigens as maternal erythrocytes. Neonatal removal of the bursa is also known to depress anaphylactic reactions (Sato and Glick, 1965).

Although bursectomy suppresses the immune response to Plasmodium lophurae infection in the chicken (Longenecker et al., 1966), it seems to have little effect on avian coccidiosis (Pierce and Long, 1965; Rose, 1968; Rose and Long, 1970). Challey (1962) suggested that the greater mortality he observed in bursectomized chickens infected with Eimeria tenella might be due to an impairment of the immune response.

Suppression of bursal development and consequent suppression of humoral antibody production can be achieved by the injection of 19-nortestosterone into developing embryos on the fifth day of incubation (Meyer et al., 1959; Mueller et al., 1960; Papermaster et al., 1961; Papermaster and Good, 1962; Claflin et al., 1966), or by the injection of testosterone propionate on the 12th day of embryonic development (Auerbach, 1962; Carey and Warner, 1964; Warner et al., 1969). These treatments, however, produce secondary complications on the rectal complex: stunted growth of the ventral anal lip, an increased musculature of the dorsal lip and an attenuated proctodeal canal with very few anal mucoid glands. Such morphological alterations severely affect defaecation and greatly increase problems in the husbandry of the treated birds.

Chickens subjected to hormonal bursectomy also show atrophy of the thymic cortex and a reduction in the number and size of splenic nodules. Pierce and Long (1965) noticed very few pyroninophilic cells in the spleen and caeca of hormonally bursectomized chickens; the caecal lymphoid tissue was either not detected, or was very much reduced. In hormonally bursectomized (HBx) fowls with normal thymuses, the lymphoid follicles in the spleen are normal but the amount of non-nodular lymphoid tissue is considerably reduced (Isaković and Janković, 1964). In

bursectomized birds with complete atrophy of the thymic cortex, splenic atrophy is extreme: lymphoid follicles are absent, lymphoid cells are scarce, and only reticular-type cells are present in normal numbers. These observations indicate a correlation between the integrity of the thymic cortex and the development of lymphoid follicles in the spleen. Although antibody production is depressed below detectable levels in HBx chickens, the production of plasma cells and non-antibody gamma globulin still takes place (Carey and Warner, 1964). Claflin et al. (1966) have indicated that a depression in the largely bursa-dependent early antibody (IgM) response determined the later more severe impairment of IgG immunoglobulin synthesis.

Antibody production has also been suppressed in chickens by the surgical removal of the spleen at 17 weeks of age (Rosenquist and Wolfe, 1962). These investigators suggested that such a depression, achieved only in the older animals, was probably due to the inability of the adults to compensate as well as the younger animals for the loss of such a large lymphoid organ. Many of the splenectomized animals showed a lag in antibody synthesis, indicating that the spleen may normally synthesize antibodies earlier than other antibody-forming tissues. Such an early synthesis by the spleen was ascribed by these authors to the presence of a greater number of

immunologically competent cells or to a more effective uptake of antigen by this organ.

As in the case of bursectomy, splenectomy also produces an impairment in immune reactions against Plasmodium infection in birds (Manwell et al., 1957; Longenecker et al., 1966), but not against infection by Eimeria tenella (Rose, 1968). The importance of the spleen in the immunity to malaria might be due to the fact that splenic macrophages play an important role in host resistance to this disease (Taliaferro, 1956).

(iii) Antilymphocyte serum

In recent years there has been increasing interest in the use of antilymphocyte serum (ALS) as an immunosuppressive agent; remarkable results have been reported in the suppression of immune reactions to allografts and xenografts. Such serum treatment, when combined with thymectomy, has resulted in a more sustained suppression of allograft rejection than has been possible with other methods (Monaco et al., 1965a, 1965b; Jeejeebhoy, 1965). Although ALS has the potential for a specificity not attainable with immunosuppressive drugs, radiation or surgery, the specificity of this serum is not absolute in that the whole immune mechanism is affected and the resistance of the recipient to infection is also greatly decreased.

A striking instance of inhibition of allograft rejection in rats by ALS was reported in 1963 by Woodruff and Anderson. They showed that daily administration of 2 ml. of the antiserum for one week before grafting, followed by daily administration of 1 ml. for two weeks after transplantation, produced an increase in mean survival of the graft from eight days in untreated controls to 28 days in the treated group. Recent experiments in mice by Monaco et al. (1966) indicated the extraordinary immunosuppressive potential of ALS prepared in rabbits. Such serum caused a prolongation, not only of allografts across the strong H-2 histocompatibility barrier, but also across the species barrier in grafts of rat skin to mouse. However, the effect of antilymphocytic serum on skin grafts between randomly-bred guinea pigs has not been impressive, only slight delays being recorded by these latter authors in the rejection of first-set grafts in serum-treated recipients.

ALS is also found to be effective in suppressing hypersensitivity reactions. Waksman and Arbouys (1960) and Waksman et al (1961) successfully used ALS to suppress two types of delayed reactions: tuberculin sensitivity and contact sensitivity to dinitrochlorobenzene. In addition, Currey and Ziff (1968) demonstrated an anti-inflammatory effect for the gamma globulin fraction of

ALS. Antilymphocyte serum also has an inhibitory effect on the immune response of chickens against coccidial infection (Euzeby et al., 1969).

The 7S gamma globulin fraction is responsible for the antilymphocytic activity of ALS (James and Anderson, 1967). Denman and Frenkel (1967a) showed that when rats were rendered tolerant to normal rabbit IgG (NRG), they required smaller and less prolonged injections of rabbit antilymphocyte gamma globulin (ALG) to induce suppression of immunity. Histologically, these rats demonstrated a severe depletion of lymphocytes when compared to rats not made tolerant to NRG before being subjected to the ALG treatment.

The primary effect of ALS is believed to be on the peripheral lymphocytes such as those in the thoracic duct (Levey and Medawar, 1967). Most of the small lymphocytes have a long life span, are immunologically competent and may be derived from the thymus (Miller and Osoba, 1967). Martin and Miller (1967) showed that ALS destroyed these long-lived small lymphocytes and also stimulated some of them to enlarge into immature-appearing blast forms. They also noticed that the number of small lymphocytes in the blood returned to normal levels after the treatment was discontinued, whereas the number of small cells in the thoracic duct lymph remained depressed for at least six

weeks after ALS administration. Since these long-lived small lymphocytes are known to be capable of initiating the homograft response (Gowans and McGregor, 1965), it has been suggested that destruction of these cells could account for long graft survival in ALS-treated rats (Monaco et al., 1966; Abaza et al., 1966; Anderson et al., 1967; Schwarz et al., 1968). The long-lived small lymphocytes may also play a role in the initiation of primary humoral responses (Gowans et al., 1962; Currey and Ziff, 1968) which can also be depressed by antilymphocyte serum (James and Anderson, 1967; James and Jubb, 1967).

The effect of ALS on the central lymphoid organs may be indirect only in the sense that they liberate the susceptible lymphoid cells into the peripheral circulation (Levey and Medawar, 1967). For example, Barth (1969) suggested a preferential effect of ALS on antigen-sensitive, thymus-derived cells. Iwasaki et al. (1967) showed that in the lymphoid tissues of ALS-treated animals, small lymphocytes were replaced by large and medium-sized proliferating cells with pyroninophilic cytoplasm; germinal centers were also formed in these animals. These findings are in accordance with the hypothesis of Levey and Medawar (1967), that at least one action of antilymphocytic agents is to stimulate the transformation of lymphocytes into blast cells and that its immunosuppressive effect is not necessarily dependent upon a lymphocytotoxic action.

Antiserum prepared against thymus cells (ATS) is more potent than that prepared against peripheral lymphocytes (Nagaya and Sieker, 1965). These authors believe that such a discrepancy may be due to the ability of ATS to suppress the proliferation of thymic cortical lymphocytes, probably by inactivating a thymic humoral factor. Following ATS-treatment, there is a compensating increase in the production of a thymic humoral factor, accounting for the eventual recovery of immunologic potential when the treatment is discontinued.

The advantage of specific adsorption of antilymphocytic serum has been shown by Asakuma and Reif (1968) who found that unadsorbed antisera prepared against splenic lymphocytes had no specific activity against different types of lymphocytes and that antibody activity against thymocytes could be removed specifically by adsorption with thymocytes.

(iv) Antimetabolites of the purine bases

Suppression of immunity has also been accomplished in the past by the use of a number of chemical compounds including benzene, toluene and the salicylates. Other immunosuppressive agents include alkylating agents, folic acid antagonists, antimetabolites of the purine bases, analogues of the pyrimidine bases and plant alkaloids

(reviewed by Gabrielsen and Good, 1967). Chemical immunosuppression may affect one or more phases of the immune response, from the initial processing of the antigen by cells of the reticuloendothelial system, through the steps of the adaptive process involving cell differentiation and the effector stage.

The purine analogues block the synthesis of nucleic acids by competitive inhibition (Berenbaum, 1965) and hence protein synthesis and cell differentiation as well. They have been shown by several workers (Nathan et al., 1961; Orbach-Arbouys and Eyquem, 1961; Rubin and Lewis, 1961; Frisch et al., 1962; Hoyer et al., 1962; Laplante et al., 1962; Page et al., 1962; Borel and Schwartz, 1964; Rubenstein and Wolff, 1964) to suppress a number of immune reactions including delayed hypersensitivity and allograft rejection.

Azathioprine [6-(1-methyl-4-nitro-5-imidazolyl) thiopurine], the imidazols derivative of 6-mercaptopurine (6-MP), commercially known as "Imuran" was introduced in 1961 by Elion, Rundles and their associates, and has largely eclipsed the original 6-MP in clinical use. The clearest advantage of azathioprine over 6-MP is the fact that the former does not seem to damage the epithelium of the gut, which is a serious disadvantage with the administration of 6-MP (Elion et al., 1961). Azathioprine is also

characterized by low toxicity; the single dose LD_{50} for rats is 310 mg/Kg i.p. and 400 mg/Kg *per os* (Elion *et al.*, 1961). Metabolic studies have shown that azathioprine is absorbed well from the intestinal tract and that it is converted *in vivo* in mammals to 6-MP and its oxidation product 6-thiouric acid.

Elion and Hitchings in their recent review (1965) have cited a series of renal allograft experiments in dogs, in support for the claim that azathioprine is an effective suppressant of the homograft response. Furthermore, Preston *et al.* (1965) and Assimacopoulos and Salmon (1965) have observed prolonged survival of jejunum and stomach allografts in dogs treated with this drug. Azathioprine is also capable of suppressing the formation of antibodies in mice against sheep red cells (Nathan *et al.*, 1961). On the other hand, this compound has no effect on the auto-immune disease ulcerative colitis (Bowen *et al.*, 1966).

(v) Adrenal steroid hormones

It has long been known that adrenal steroids inhibit cellular proliferation in different tissues and that these compounds are lympholytic with a particular affinity for thymic cells (Dougherty, 1952a). As far as thymic cells are concerned, lympholytic activity is greatest for cortisol and next in potency are cortisone, corticosterone

and 11-hydrocortisone. It has been conclusively demonstrated that cortisone does not affect the lymphocytes until it is reduced *in vivo* to cortisol, which causes the disintegration of the lymphocyte nucleus or lymphokaryorrhexis (Dougherty *et al.*, 1964).

There are three ways by which corticosteroids bring about an involution of lymphatic tissue: by stimulating lymphokaryorrhexis (LCK), by inhibiting mitosis at the metaphase stage and by inhibiting the synthesis of DNA. In the thymus, the LCK effect begins shortly after hormone administration; this effect can also be demonstrated in the spleen, lymph nodes, Peyer's patches and lymphatic tissue aggregates in the intestine, as well as in lymphocytes of the blood (Frank and Dougherty, 1953). The small lymphocytes tend to disintegrate more rapidly than the larger ones, although the large and medium-sized lymphocytes also shed their cytoplasm and reveal nuclear shrinkage (Dougherty *et al.*, 1964). Following the loss of lymphocytes, the lymphatic tissue degenerates extremely rapidly (Frank *et al.*, 1953). Reticuloendothelial cells and reticular lymphocytes resistant to the disintegrating action of coticosteroids provide a seed bed of progenitor cells which can effect the repopulation of lymphocytes after corticosteroid levels decrease.

There has been an extensive series of experiments indicating the extraordinary potency of adrenal steroids in preventing antibody production and antibody-mediated hypersensitivity reactions. Billingham and co-workers (1951) reported that parenteral administration of cortisone lengthened the life of skin homografts in rabbits by a factor of 3 to 4; similar effects were also obtained by the local application of the hormone in the graft bed itself. Syverton et al. (1952) found a higher percentage of graft "takes" in X-irradiated animals treated with cortisone than in animals subjected to irradiation alone. Corticosterone has also been found to be effective in maintaining the integrity of homografts in chickens (Aspinall and Meyer, 1964). These latter authors suggest that the steroid either blocks the thymus-dependent skin homograft immune mechanisms or prolongs the graft survival non-specifically through its anti-inflammatory activity. Systemic anaphylaxis in mice is also found to be inhibited by treatment with cortisone and related agents (Solotorovsky and Winsten, 1954; Cameron, 1957). In general, the adrenal steroids can affect antibody-mediated hypersensitivity in two ways: (1) by totally suppressing the production of antibody if administered before or at the time of sensitization and (2) by modifying the local or systemic response in animals actively or passively sensitized before the drug

is given. The second effect of the adrenal corticosteroids is dependent on several factors: the species of animal used, the steroid agent employed, the dosage, the route and time of administration, and the amount of antibody which has already been produced by the animal at the time of treatment.

Lately, there have been some attempts to suppress the immunity to coccidia in the fowl by corticosteroid treatment. Cortisone acetate is ineffective in the suppression of the immune response against Eimeria brunetti (Rose and Long, 1970). McLoughlin (1969), however, reported the successful transmission of E. meleagrimitis, a species normally parasitic only in the turkey, to dexamethasone- (a corticosteroid) treated chickens; the chicken-specific E. tenella, on the other hand, failed to develop in dexamethasone-treated turkeys. Corticosteroid treatment affects the site specificity of E. tenella; dexamethasone produces an ectopic development of the schizogonic stages of this species in the mid-intestine of orally infected birds and in the liver of chickens intravenously inoculated with sporozoites (Long, 1970). The results of Rose (1970) have shown that cortisone treatment of chickens affected their acquired resistance against Eimeria mivati and also prolonged the patent period of initial infections; these chickens also produced a greater

number of oöcysts than untreated control birds. She suggested that the prolonged patent period resulted from additional generations of schizogony that occurred due to the immunosuppressive effect of cortisone. Schizonts were seen in sections of the intestine of treated birds on the 24th day after infection while, in the untreated controls, schizonts were absent after the eighth day post-infection.

(vi) Irradiation

Of all the methods proposed to depress immunological responses none has been more widely used than X-irradiation; this is probably due to its effectiveness and the ease with which it can be applied. Total body irradiation at sub-lethal doses often decreases host immunity to specific infections and reduces the ability of animals to produce antibody. Effective suppression of immunity is achieved only with "hard" rays such as X- and gamma-rays since the penetrating power of alpha and beta rays (soft rays) is very limited. The effect of hard rays is accomplished by the liberation of photo-electrons in the tissues as a result of bombardment by these high energy electromagnetic waves. The amount of radiation generated by an X- or gamma-ray source is usually measured in roentgen (r) units, a unit based on Roentgen's original description of the ability of X-rays to ionize air. The energy dissipated by

1 r corresponds to 84 ergs per gram tissue; another unit, the rad (R), is defined as that dose of any ionizing radiation which results in the emission of 100 ergs of energy per gram tissue. Gamma-rays have a shorter wavelength than X-rays and are usually found associated with beta rays.

In laboratory animals the organs most highly sensitive to radiation are the gonads, bone marrow, spleen, lymph nodes, thymus, parts of the gastrointenstinal tract, skin and bone. Nerve, muscle and most exocrine glands are relatively radioresistant (Bloom and Jacobson, 1948). A few days after the administration of an LD_{50} dose of radiation, the blood-forming organs become markedly reduced in size and the bone marrow and lymph nodes become aplastic. In the course of one to two weeks after exposure to radiation, the haematopoietic organs gradually regain their activity; however, complete recovery of the bone marrow function is rarely achieved. The lymph nodes, the thymus and the spleen can return completely to normal function, but only several months after treatment.

It is, of course, the damage or death of the lymphoid tissue which is responsible for the impairment of immune mechanisms. Murray (1948) found that, although the reticular cells and fixed macrophages were not greatly injured or killed in the spleen of rabbits irradiated with

400 to 800 r, the lymphoid cells showed abnormal clumping and almost all medium-sized lymphocytes were eliminated within a few hours after treatment. The plasma cells on the other hand, were relatively radioresistant. Radiation also retards the rate of digestion of phagocytosed materials (Donaldson et al., 1956). Circulating leucocytes are affected by very low doses: 800 r causes a 90 per cent reduction in lymphocytes (after 72 hours) and heterophils (after 96 hours) in the rabbit. Recovery to normal limits requires 50 days for lymphocytes but only 23 days for heterophils (Jacobson, 1954).

It has been suggested (Taliaferro et al., 1964) that the chief damage produced by irradiation is to the genetic information necessary to produce antibody and to the proliferation and maturation of antibody-producing cells. The inductive phase of antibody formation during the first 12 hours of the latent period is highly radiosensitive. This preliminary event or series of events is highly important, since it determines whether antibody will be formed and, roughly, how much of it will be synthesized. The second part of the latent period, when antibody-forming cells are proliferating and maturing and the new synthetic machinery is developing, is also highly radiosensitive. The third phase of antibody production, when proteins are being actively synthesized, is much less

affected by irradiation than the two preceding ones (Taliaferro et al., 1964). The second part of the inductive phase seems to be associated with immature, rapidly dividing hemocytoblasts, whereas the phase of active protein synthesis is associated with the more mature plasma cells which divide infrequently, or not at all.

Once formed, the antibody molecule is not denatured or modified by ionizing radiation within the sublethal dose range (Taliaferro et al., 1964). Burrows et al. (1950a) indicated that low doses of irradiation with 100 and 150 r following the immunization of guinea pigs did not appreciably affect the serum antibody titer, but produced partial or complete inhibition of the excretion of faecal and urinary antibodies. According to Sahiar and Schwartz (1965) and Nettesheim et al. (1969), heavily X-irradiated rabbits showed a marked prolongation of 19S antibody formation in addition to the suppression of 7S antibody synthesis. The inhibitory effect of a single dose of radiation on 7S gamma globulin lasts about 30 days (Robbins et al., 1963). In the case of hemolysin production of the rabbit, X-irradiation produces only a transient depression (Taliaferro and Taliaferro, 1964), and recovery starts 28 days after treatment.

There have been several reports on the effect of radiation on homograft rejection. Toolan (1953)

demonstrated a temporary effect of X-irradiation on the rejection of heterologous transplants in mice. Main and Prehn (1955) noted that the immunosuppressive effect of irradiation on homograft rejection is temporary, lasting for a period of less than four weeks. Clemmensen (1937) and Dempster et al. (1950) have indicated that the effect of X-irradiation on heterologous grafting is merely to delay the immune response. These authors suggested that there might be two factors involved in the host reaction: (1) a highly radiosensitive receptor which initiates the immune response and (2) a radioresistant effector which manufactures the antibody. The radiosusceptibility of the two stages postulated here is analogous to that observed in humoral antibody synthesis.

Irradiation has also been shown to have an effect on hypersensitivity reactions. For example, the inflammatory response to a subcutaneous injection of ovalbumin mixed with India ink is much less intense in mice irradiated with doses greater than 900 r than in non-irradiated controls (Townsend and Campbell, 1949). In rabbits 300 r does not appreciably alter cell-mediated hypersensitivity reactions when diphtheria toxoid is injected in the time period of three days before to 18 days after irradiation; the Arthus phenomenon is delayed by about a week in its appearance when the toxoid is

injected three hours before or 18 hours after X-ray treatment (Salvin and Smith, 1959). An 800 r dose of X-rays inhibits delayed skin reactivity to Mycobacterium butyricum in rabbits (Uhr and Scharff, 1960). Brooke (1962) also showed that this hypersensitivity can be markedly depressed for more than six weeks in rabbits by irradiation with 400 r of X-rays. Irradiation also accelerates the damaging effects of pathogenic organisms (Burrows et al., 1950); the intensity of experimental enteric cholera infection is much greater in guinea pigs after treatment with 150-200 r. The loss of "innate immunity" following irradiation is most easily detected in low-level infections where the immunity is high (Taliaferro et al., 1964). Gallily and Feldman (1967) suggested that the depressed immunological reactivity of animals exposed to sublethal doses of X-rays could be partly due to the impaired capacity of macrophages of irradiated animals to process antigenic material. Rose and Long (1970) obtained inconsistent results following primary infections of neonatally irradiated chickens with two species of Eimeria. The treatment appeared to enhance oöcyst production in 5- and 8 1/2-week-old fowls infected with E. brunetti; there was, however, decreased oöcyst output in two-week-old chickens infected with the same species and in three- and five-week-old fowls infected with E. maxima.

III. MATERIALS AND METHODS

A. Chickens

The Macdonald College M-3 inbred line[1] of White Leghorn chickens was used throughout this investigation. The donors of skin homotransplants belonged to an inbred line of black-feathered "Laflèche" strain[2] or to a mutant strain of brown-feathered chickens. All fowls were obtained from the Animal Science Department of Macdonald College and were hatched in their incubators. The birds were reared on coccidiostat-free "Supersweet" chick starter feed and water ad libitum. All the experiments were carried out on three-week-old chickens selected at random. The husbandry of these fowls was usually uncomplicated except after hormonal bursectomy (see below); the cloacal plugging that occurred in these birds was reduced to a minimum by feeding them on very moist chick starter feed.

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[1]This line has been maintained as a closed flock, in the Animal Science Department, since it was started in 1959 with 13 hens and one cock.

[2]This strain of chickens has been maintained as a closed population for at least four generations.

B. Neonatal thymectomy

The procedure used here was essentially that of Aspinall *et al.* (1963). The thymic lobes were surgically removed under "Nembutal" (Abbott Laboratories) anesthesia within 24 hours after hatching. The chick to be operated was placed on its back on a perforated "Plexiglass" operating board, and restrained with strings looped around the legs, wings and neck; the strings were held in place by means of springs hooked onto the perforations in the operating board.

A longitudinal incision was made in the mid-ventral region along the entire length of the neck, extending to the anterior tip of the sternum, and the flaps of skin were retracted by means of surgical clamps. The thymic lobes were clearly visible under a magnifying lens. The lobes were easily detached with a pipette attached to a water-driven aspirator. The wound was then closed with surgical skin clips. By this method it was possible to remove from 95 to 100 per cent of the thymic tissue. Virtually no bleeding occurred and with some practice this operation could be performed within ten minutes. Mortality was almost nil.

C. Surgical bursectomy

The bursa was surgically removed within 48 hours after hatching, according to the method described by Mueller et al. (1960). The anesthetized chick was placed on the operating board with the ventral side down and restrained by means of strings and clamps, as mentioned above. The uropygium was retracted with the help of surgical clamps and a transverse incision was made at its base above the cloacal opening. The bursa was easily accessible through the incision and with the help of a pair of forceps and fine curved scissors this organ was excised close to its base, care being taken not to cut the large blood vessels in the vicinity. This operation was relatively easy to perform. The skin was closed with suturing thread; wound clips were not found suitable since they tended to interfere with muscle movements during defaecation.

D. Surgical splenectomy

The anesthetized chick was placed on the operating table on its back and restrained; an oblique incision was then made on the left ventral side of the body immediately below the rib cage. The spleen was visible beneath the junction of the proventriculus and the gizzard. A ligature

was made at the base of the organ and the splenic tissue was removed with a fine pair of surgical scissors. The body wall was then sutured with fine surgical gut and the incision in the skin was closed with stainless steel wound clips. This operation was difficult to perform due to the deep-seated location of the spleen; heavy bleeding occurred frequently, resulting in a high rate of mortality among splenectomized animals. In addition, the softness of the splenic tissue made it difficult to grasp the organ with forceps, thus complicating its excision.

E. Hormonal bursectomy

This operation was done in the first experiments by injecting 0.63 mg of 19-nortestosterone (Nutritional Biochemicals Corporation) in 0.1 ml domestic corn oil into the albumen of the egg on the 5th day of incubation. Although this treatment resulted in the complete suppression of bursal development, it also caused heavy mortality among the treated chickens due to plugging of the cloaca. In later experiments the method of Warner *et al.* (1962) was used; this technique involved the injection of 3.5 mg of testosterone propionate (Matheson, Coleman and Bell) in 0.1 ml corn oil into the allantoic cavity of the embryo on the 12th day of incubation. For details of the technique please see Beveridge and Burnet (1946). This treatment

also resulted in the complete inhibition of bursal development but with the advantage that the secondary effects on the rectal complex were less severe than in the previous technique.

F. Imuran treatment

Imuran (azathioprine) in powder form was obtained as a gift of Burroughs Wellcome and Co. (Canada) Ltd. It was administered orally in gelatin capsules or injected intraperitoneally in 0.01 N sodium hydroxide solution as recommended by Elion et al. (1961). In all cases, treatment was started on the day before antigen administration and continued daily until the experiment was terminated. For oral administration of the drug, arbitrary doses were used, ranging from 10 mg. to 160 mg. per kilogram body weight; much smaller doses were used for intraperitoneal injections.

G. Cortisone acetate treatment

Cortisone acetate (Nutritional Biochemicals Corporation) was used either to inhibit thymic development in the embryo or as an immunosuppressant in three-week-old chickens. A dose of 0.1 mg. was used for injection into the egg either in 0.1 ml corn oil or dissolved in an equal

volume of 50% ethyl alcohol. All prenatal injections were made into the yolk sac of the embryo (Beveridge and Burnet, 1946). This hormone was administered at a dose of 20 mg/Kg body weight in 0.1 ml corn oil for treatment of three-week-old birds; the injections were done intraperitoneally, beginning on the day before antigen administration and continuing daily until the experiment was completed.

H. Preparation of antilymphocyte sera

(i) General procedure

Antisera were prepared in rabbits by the injection of cells from the thymus, bursa or spleen of three-week-old chickens. All manipulations were carried out under aseptic conditions. The glands were removed from the birds and transferred to sterile Hanks' balanced salt solution. The tissues were cut into small pieces and placed in a small volume of Hanks' solution on a 50 mesh/cm stainless steel wire gauze lining the inner surface of a small Petri dish. The cells were separated by grinding the tissue through the wire mesh with a sterile glass pestle. The mesh was removed from the Petri dish and the cell suspension was filtered through a double layer of cotton gauze into a sterile centrifuge tube kept in an ice bath. The cells were then counted in a hemacytometer.

Rabbits were immunized by three intravenous injections, each consisting of approximately 1 x 10^9 cells suspended in sterile Hanks' solution; the injections were done 14 days apart and the animals were bled by cardiac puncture seven days after the last injection (Miller, 1968). Four batches of antilymphocyte sera (ALS) were used in this investigation. These were prepared against either thymus (ATS) or bursa (ABS) cells. All of these and the pre-immunization normal rabbit sera were fractionated by diethylaminoethyl-(DEAE) cellulose column chromatography. The mean leucocyte agglutination titer (Greaves et al., 1969) of the ATS used in series D was 1:69 and that of the ABS was 1:32.

(ii) Column chromatography

Standard DEAE-cellulose (capacity of 0.89 meq/gm), purchased from the Carl Schleicher and Schuell Co., Keene, N.H., U.S.A., was prepared for use as described by Peterson and Sober (1962). Isolation of the serum gamma globulin was done as recommended by Fahey et al. (1958).

The antilymphocyte sera used in experimental series C contained three rabbit antithymus and two rabbit anti-bursa sera. Those used in experimental series D included three antithymus and three antibursa sera. Each of these sera was purified by precipitation at 50% ammonium sulfate

The electrophoretic and immunoelectrophoretic patterns of globulin fractions

Figure 1. A. Anti-thymus gamma globulin (Series C, Experiment II)

B. Anti-bursa gamma globulin (Series C, Experiment II)

Figure 2. Antithymus globulin (Series D, Experiment II)

Figure 3. Homologous normal rabbit globulin used in antithymus globulin-treated groups (Series D, Experiment II)

Figure 4. Antibursa globulin (Series D, Experiment II)

Figure 5. Homologous normal rabbit globulin used in antibursa globulin-treated groups (Series D, Experiment II)

Legend

a	alpha globulin
al	albumin
b	beta globulin
g	gamma globulin

Fig. 1

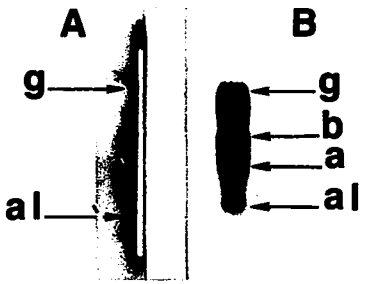

Fig. 2

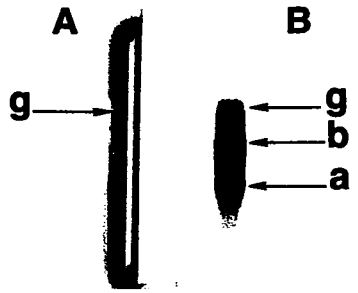

Fig. 3

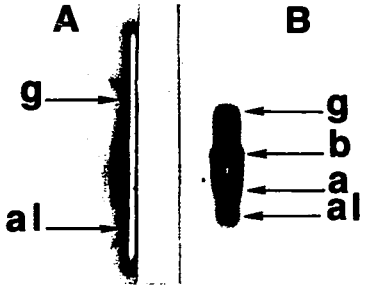

Fig. 4

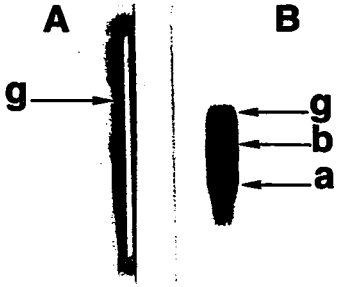

Fig. 5

saturation (Kabat and Mayer, 1961), concentrated to 50 ml by pervaporation and dialysis, and then chromatographed through 2.5^{cm} x 110^{cm} DEAE-columns.

A globulin fraction was eluted from each serum with 0.01 M phosphate buffer of pH 8.0. The gamma globulins of three antithymus and two antibursa sera were pooled separately, concentrated to 50 ml and re-chromatographed through other 2.5^{cm} x 110^{cm} DEAE-columns. The electrophoretic and immunoelectrophoretic analyses of the antilymphocyte globulins (ALG) eluted with 0.01 M phosphate buffer are illustrated in Figures 1, 2 and 4.

The pre-immunization sera obtained from each of the nine rabbits used for the preparation of the ATS and ABS used in experimental series D were also pooled separately into the two corresponding groups and purified as above by ammonium sulfate and DEAE-column chromatography. The electrophoretic analyses of these fractions are shown in Figures 3 and 5.

(iii) Adsorption of the antilymphocyte globulins

Batches of purified ALG used in experimental series C were adsorbed with thymus and/or bursa cells; both adsorbed and unadsorbed antisera were used in this investigation. Adsorption was carried out by mixing

1×10^8 cells with 1 ml of ALG and then incubating the mixture at 5^oC for 18 hours. The cells were later removed by centrifugation in the cold; all antisera were then stored aseptically at 5^oC.

I. Gamma irradiation

Irradiation was carried out with a cobalt 60 source in an Atomic Energy of Canada Ltd. "Gamma cell 220," at a dose rate of approximately 0.75 m rads/hr. The low doses necessary for the present study were obtained by shielding the source with a 50 per cent or 70 per cent lead attenuator. Neonatal birds were given whole body irradiation within 24 to 48 hours of hatching; whole body irradiation of three-week-old birds was performed on the day before antigen administration. The sublethal dose for neonatal chicks was found to be approximately 700R whereas three-week-old fowls tolerated doses up to 800R. The sublethal dose of gamma radiation for three-week-old neonatally thymectomized birds was about 750R.

J. Hemagglutination tests

One-tenth ml of 5% sheep red blood cells (Srbc) suspended in pH 7.2 phosphate buffered saline was injected intravenously into the wing vein with a 26-gauge needle.

Exsanguination was carried out a week later, either through the wing vein or by cardiac puncture. In the latter technique, the best results were obtained when a small incision was made on the ventral side of the anesthetized animal anterior to the heart and blood collected from the heart at the base of the carotid artery; bleeding was done with a 22-gauge needle. The incision was then closed with wound clips. Up to 10 ml of blood could be collected by this technique; however, some mortality occurred. Bleeding through the wing vein provided only 2-3 ml, but did not produce any mortality.

All sera were stored in the freezer at -20°C before hemagglutination tests were performed. The titer was determined by direct hemagglutination in "Microtiter" agglutination plates (Cooke Engineering Company, Alexandria, Virginia, U.S.A.). Halving dilutions of inactivated sera were made in saline in the wells of the agglutination plate; 50 µl of 2.5% Srbc in saline was then added to each serum dilution, the reagents were mixed and allowed to react overnight at room temperature. The agglutination titer was scored from 0 to 4+ (Stavitzky, 1954). The titer was read as the highest dilution of the serum showing a 1+ reaction.

Indirect passive hemagglutination test was done essentially as described by Stavitzky (1954). Sheep cells

were tanned with 1:40,000 tannic acid in pH 7.2 phosphate buffered saline and then sensitized at pH 6.4 with 1.5 mg/ml protein. The unknown sera were inactivated and adsorbed with an equal volume of packed sheep rbc before use. Halving serum dilutions were made in 50 µl of saline and then 25 µl of tanned sensitized 2.5% sheep red cell suspension was added to each dilution. The reagents were mixed and incubated at room temperature overnight. The titer was taken as the highest dilution giving a 1+ reaction.

K. Skin transplantation

All chickens to be skin-grafted were "debeaked" by cauterization on the day before the operation to prevent destruction of the graft by pecking. Both donor and recipient chickens were anesthetized with "Nembutal" and the feathers were plucked from a $50^{mm} \times 25^{mm}$ area in the mid-dorsal region. Both chickens were then placed on the operating table with their ventral side down and restrained as described above. In homotransplantation, a rectangular piece of skin approximately $15^{mm} \times 10^{mm}$ in size was removed from the recipient's back; this skin was then replaced by a similar piece from the same area of the donor chicken. Before placing the graft, the area was cleared of blood clots and fatty tissue.

In chickens which received both auto- and homografts, a rectangular piece of skin about $26^{mm} \times 10^{mm}$ in size was excised from the mid-dorsal region of the recipient; it was then cut into two equal pieces, each measuring approximately $13^{mm} \times 10^{mm}$. The orientation of the feather follicles of the anterior piece was then reversed and placed in the same area; the posterior half was replaced by a similar piece of skin from the donor bird. The orientation of the feather growth in the homograft was not reversed since growth of the dark donor feathers would indicate acceptance of the graft. The grafts were kept in place by sutures made first at the corners, followed by a continuous type suture along the periphery.

The sutures were removed within two weeks after the operation, taking care not to apply undue pressure and cause only minimum disturbance in the contact between the grafted skin and the underlying host tissue. The condition of the transplant was checked once a week until sacrifice. Frequently, the grafted area became edematous and the homografts showed discoloration followed by necrosis. The autografts did not normally undergo such discoloration; in these the epidermis peeled off within two weeks postoperatively, and feather growth usually started by about three weeks. The homograft usually remained intact for

about two weeks and in most cases then became necrotic, followed by peeling of the entire thickness of the grafted skin. In homografts that were "taken," donor feather growth started about four weeks after the operation and usually the feathers remained intact until sacrifice. The growth of donor feathers was the chief criterion used to designate the homograft as "accepted" or "rejected."

L. Coccidial infection

(i) General procedure

All infections with Eimeria tenella were carried out orally; the required number of sporulated oöcysts were suspended in 1 ml saline and administered to each bird with a Pasteur pipette. Infected fowls belonging to each treatment group were kept together in 22" x 20" x 15" galvanized steel isolation cages in a fly-screened enclosure far removed from the uninfected groups; this enclosure was entered through double doors. The droppings from each cage were collected daily on a plastic sheet.

(ii) Oöcyst collection

A modified version of the centrifugal flotation technique of Faust et al. (1939) was used for the recovery of oöcysts from faeces. Droppings accumulated over a 24-hr

period were collected from the bottom of each cage and mixed with sufficient water to form a liquid suspension. After thoroughly mixing the suspension, it was strained through a double layer of cotton gauze and centrifuged at a speed of approximately 1000 x g for ten minutes. The sediment was resuspended in a saturated sodium chloride solution having a specific gravity of approximately 1.18 and again centrifuged as before in a graduated centrifuge tube. The oöcysts remained suspended in the supernatant; the number of oöcysts in this known volume was determined with the help of a McMaster nematode egg counting chamber (Soulsby, 1965). The total number of oöcysts present per milliliter of the suspension was obtained from at least three counts; the number of oöcysts produced per chicken per day was calculated from this value.

For the isolation of oöcysts, the final supernatant fluid containing a known number of oöcysts per volume was diluted in a liter of tap water and left undisturbed overnight in a glass cylinder. The next day most of the supernatant water was removed by aspiration, to leave about 100 ml in the bottom of the cylinder. This bottom layer was then mixed thoroughly and centrifuged as above; oöcysts were obtained in the sediment, free of debris. The supernatant fluid from the centrifugation was discarded and the oöcysts were resuspended in 2% potassium dichromate

solution in distilled water (Joyner, 1958); they were then transferred to 50 ml Erlenmeyer flasks and left at room temperature. By 48 hours most of the oöcysts had undergone sporulation. These oöcysts were then stored in the same dichromate solution at $5^{o}C$; under these conditions the oöcysts remained infective for about three weeks. The oöcysts were washed at least five times in water and counted before administering to the experimental chickens.

(iii) Histology

Histological investigations were carried out on sections of tissue from the small intestine and the caecum. The small intestine between the duodenum and the caecal junction was divided into two equal sections (SI and SII). Small rectangular pieces about $4^{mm} \times 3^{mm}$ in size were removed from the duodenum, SI, SII and caeca. They were fixed in Zenker's fluid, embedded in paraffin wax and sectioned at 8 μ; Ehrlich's hematoxylin and eosin were used for staining.

M. Statistical analysis

The analysis of variance (Moroney, 1960) and Duncan's new multiple range test (Steel and Torrie, 1960) were used to help analyze the data. When a number of mean

values were to be compared, the "F" values were estimated by the analysis of variance and when this was above the 5.0% level, the mean value of each group was compared with that of its control by Duncan's test.

IV. THE EXPERIMENTS

SERIES A

STUDIES ON THE SUPPRESSION OF HUMORAL IMMUNE MECHANISMS

Despite the fact that a great deal of work has been done in the mammalian system, data on the suppression of circulating antibody production in birds is relatively limited. Removal of the bursa of Fabricius has been the technique most effectively used for the suppression of humoral antibody reaction in the chicken. Even this treatment, however, does not produce an absolute inhibition of immunoglobulin synthesis (Claflin et al., 1966). The purpose of the present investigation was to apply some of the conventional immunosuppressive techniques which have been commonly used in mammals, to the avian system. The methods employed here included the removal of lymphoid organs and treatments with cortisone acetate, azathioprine and various doses of gamma irradiation. The effectiveness of the treatment was determined by the measurement of immunological competence to sheep red blood cells.

Experiment I

In this preliminary experiment, the effects of neonatal thymectomy (NTx), hormonal (HBx) or neonatal (NBx) bursectomy and of neonatal splenectomy (NSx) on the synthesis of anti-sheep erythrocyte hemagglutinins were investigated. Three types of controls were included in the protocol of the experiment: untreated birds injected with the sheep red cell (Srbc) antigen, untreated chickens not injected with the antigen, and chicks hatched from sham-treated eggs and later injected with the antigen. The test and the appropriate control groups were treated in the egg or in the neonatal period and then injected on the third, fourth or fifth week of life with 5% Srbc. The animals were exsanguinated one week after administration of the antigen.

The results of this experiment shown in Table I (and appendix table 1.a.) indicate that HBx was the only treatment capable of suppressing the circulating antibody response to the erythrocyte antigen. This inhibition was achieved when 0.63 mg 19-nortestosterone or 3.5 mg testosterone propionate was injected into the egg on the 5th and 12th days of incubation, respectively. The mean titer of sera from birds treated with 2.5 mg testosterone propionate was not significantly different ($P \geq .05$) from that of the untreated group; this latter result was

TABLE I. The effects of neonatal thymectomy (NTx), hormonal bursectomy (HBx), neonatal bursectomy (NBx) and neonatal splenectomy (NSx) on hemagglutinin (HA) production in chickens of different ages (Series A, Experiment I)

Treatment	Original number used	Number of birds survived	Mean $\log_2$ HA titer (a)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.
NTx	15	11	1.7	4	252 ± 8.3
HBx (19 nt)	10	5	0	4	176 ± 10.7
Sham op. control	5	4	2.5	4	353 ± 16.8
Control/ag	5	5	2.0 (1)	4	243 ± 13.4
Control/no ag	4	4	0	4	242 ± 7.7
NTx	20	16	3.4	5	282 ± 12.9
HBx (19 nt)	9	3	0	5	271 ± 13.9
HBx (Tp 2.5)	10	8	0.75*	5	340 ± 31.3
HBx (Tp 3.5)	10	8	0	5	408 ± 42.8
NBx	6	3	1.0	5	320 ± 13.5
NSx	6	5	3 (1)	5	399 ± 24.8
Control	8	8	2.9	5	296 ± 14.7
Control/no ag	7	7	0	5	305 ± 14.9
NSx	6	5	1.6 (3)	6	406 ± 10.9
Control	3	3	1.3	6	369 ± 22.4

(a) titers expressed as reciprocals
* one serum in this group showed positive reaction
ag = antigen
S.E. = standard error of the mean
19 nt = 19-nortestosterone injected into the albumen
Tp 2.5 = 2.5 mg testosterone propionate injected into the allantoic cavity

Numbers in parantheses refer to the number of sera showing negative HA titer.

obtained solely because one out of the eight chickens used in this group showed a positive hemagglutinin titer.

Four out of ten splenectomized chickens used in this experiment also failed to produce antibodies to the Srbc antigen. Post mortem examination of those chickens which produced hemagglutinins revealed that the splenic tissue had undergone a considerable degree of regeneration; the mean weight of the regenerated tissue was approximately 30 per cent of that of normal birds of the same age. Natural hemagglutinins against sheep erythrocytes were not observed in chickens used in the present study; this contradicts the findings of Fine et al. (1954).

In comparison with the untreated fowls, there was a significant reduction ($P \leq .05$) in the mean body weight of 19-nortestosterone-treated chickens at four weeks of age (appendix tables 1.b. and 1.c.). This weight reduction was not consistent since the difference between the treated and control groups was not significant in five-week-old birds. None of the birds in the other treatment groups showed a significant weight reduction.

Experiment II

Since it is well established by many workers (please see Review of the Literature) that thymic influence on the secondary lymphoid tissues starts long before hatching,

neonatal thymectomy is not considered to be an effective technique for producing a substantial impairment of thymus-controlled functions. In view of this fact, an attempt was made to develop a technique which would inhibit thymic development in the prenatal period. Because lymphocyto-poiesis in this organ starts on the ninth day of embryonic development (Ackerman and Knouff, 1964), it was decided to attempt the suppression of thymic differentiation at this stage. The technique used here was that of Weston (1964); 0.1 mg of hydrocortisone sodium succinate (Sigma Chemical Co.) dissolved in 0.1 ml of either distilled water or 50% ethyl alcohol was injected into the albumen, yolk sac or allantoic cavity of the egg on the 12th day of incubation. This treatment, however, resulted in 95% mortality among the embryos and was therefore discontinued.

Cortisone acetate injections in the same 0.1 mg concentration, on the 9th, 10th, 11th, 12th or 14th day (CA_9, CA_{10}, CA_{11}, CA_{12} or CA_{14}) of embryonic development, on the other hand, resulted in a relatively low rate of mortality. Chicks hatched from CA-treated eggs were subjected to either neonatal thymectomy or neonatal bursectomy. Control groups were subjected to (1) prenatal injection of cortisone acetate, corn oil or 50% ethanol without subsequent surgery, (2) prenatal injection of corn oil or ethanol, followed by neonatal thymectomy or

TABLE II. The effects of prenatal cortisone treatment plus neonatal thymectomy or bursectomy on the production of anti-sheep red cell agglutinins (Series A, Experiment II)

Treatment	Original number used	Number of birds survived	Mean $\log_2$ HA titer (a)	Age at sacrifice (weeks)	Body wt sacrifice mean ± S
$CA_{9\ oil}$ + NTx	10	7	2.1 (3)	5	257 ± 20
Oil_9 + NTx	6	4	7.3	5	313 ± 8
Oil_9	6	2	8.0	5	264 ± 1
$CA_{9\ alc}$ + NTx	10	4	6.75	5	292 ± 17
Alc_9 + NTx	6	5	7.6	5	343 ± 42
Alc_9	6	3	6.3	5	307 ± 16
$CA_{10\ oil}$ + NTx	6	4	5.75	5	363 ± 27
$CA_{10\ oil}$	6	3	10	5	339 ± 11
$CA_{10\ alc}$ + NTx	10	6	7.3	5	371 ± 23
$CA_{10\ alc}$	6	3	9	5	415 ± 20
$CA_{11\ alc}$ + NTx	12	10	5.5	5	376 ± 16
$CA_{11\ alc}$	12	6	9	5	401 ± 26
$CA_{11\ oil}$ + NTx	6	5	9	5	385 ± 27
$CA_{11\ oil}$	6	3	10	5	425 ± 10
$CA_{12\ oil}$ + NTx	6	3	1 (2)	6	406 ± 17
$CA_{14\ oil}$ + NTx	15	10	1.6 (2)	5	253 ± 13
$CA_{9\ oil}$ + NBx	10	5	2.8	5	265 ± 8
Control	4	4	5.5	5	300 ± 17
$CA_{9\ oil}$ + NTx	10	7	2 (1)	4	254 ±
$CA_{9\ oil}$	10	7	4 (1)	4	296 ± 14
$CA_{9\ alc}$ + NTx	8	6	2.8	4	267 ±
$CA_{9\ alc}$	8	5	3	4	266 ± 2
$CA_{12\ oil}$ + NBx	6	3	3	4	210 ± 1
Control	6	6	4	4	302 ± 10

$CA_{9\ oil}$ - cortisone acetate dissolved in corn oil injected on the 9th day of incubation

$CA_{9\ alc}$ - cortisone acetate dissolved in 50% ethanol injected on the 9th day of incubation

nd - not done

For explanations of other symbols and abbreviations, please refer to the legend of Table I.

one treatment plus
roduction of anti-
Experiment II)

n log_2 titer (a)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.	Mean weight of glands at sacrifice (gm)			Sex	
			Thymus	Spleen	Bursa	♂	♀
1 (3)	5	257 ± 20.2	0.06	0.48	1.07	4	3
3	5	313 ± 8.4	0.06	0.49	1.85	3	1
0	5	264 ± 1.4	1.67	0.50	1.22	0	2
75	5	292 ± 17.9	0.05	0.41	1.49	3	1
6	5	343 ± 42.9	0.01	0.59	1.63	3	2
3	5	307 ± 16.8	2.00	1.54	1.82	0	3
75	5	363 ± 27.3	0.13	0.69	1.66	3	1
	5	339 ± 11.2	1.87	0.66	1.64	0	3
3	5	371 ± 23.7	0.10	0.72	1.80	5	1
	5	415 ± 20.4	3.14	0.89	2.71	2	1
5	5	376 ± 16.4	0.07	0.85	1.87	7	3
	5	401 ± 26.4	2.59	0.82	1.93	2	4
	5	385 ± 27.2	0.05	0.77	2.44	4	1
	5	425 ± 10.5	2.36	1.00	2.25	2	1
(2)	6	406 ± 17.3	nd	nd	nd	1	2
6 (2)	5	253 ± 12.7	0.00	0.56	1.36	3	7
8	5	265 ± 8.0	1.43	0.49	0.00	1	4
5	5	300 ± 17.1	1.80	0.57	1.87	2	2
(1)	4	254 ± 7.9	0.01	0.43	1.21	4	3
(1)	4	296 ± 14.7	1.62	0.84	1.45	2	3
8	4	267 ± 6.5	0.06	0.63	1.28	4	2
	4	266 ± 27.9	1.50	0.73	0.93	3	2
	4	210 ± 11.2	1.21	0.40	0.00	0	3
	4	302 ± 10.9	1.52	0.70	1.45	5	1

ved in corn oil
of incubation
ved in 50% ethanol
of incubation

and abbreviations,
I.

TABLE III

a. Comparison of the mean $\log_2$ hemagglutinin titers of sera from CA-treated birds by Duncan's multiple range test (Series A, Experiment II)

Treatment	Mean $\log_2$ HA titer (a)	Significant at 0.05 level	Significant at 0.01 level
$CA_{14\ oil}$ + NTx	1.6		
$CA_{9\ oil}$ + NTx	2.1		
$CA_{9\ oil}$ + NBx	2.8		
Control	5.5		
$CA_{10\ oil}$ + NTx	5.75		

b. Comparison of the mean weights of the bursa of Fabricius in CA-treated chickens

Treatment	Mean weight of bursa (gm)	Significant at 0.05 level	Significant at 0.01 level
$CA_{9\ oil}$ + NTx	1.07		
$CA_{14\ oil}$ + NTx	1.36		
$CA_{10\ oil}$	1.64		
$CA_{10\ oil}$ + NTx	1.66		
Control	1.87		
$CA_{11\ oil}$	2.25		
$CA_{11\ oil}$ + NTx	2.44		

(a) titers expressed as reciprocals

Any two means not covered by the same line are significantly different at the level indicated.

TABLE III (continued)

c. Comparison of the mean weights of the spleen in CA-treated birds

Treatment	Mean weight of spleen(gm)	Significant at 0.05 level	Significant at 0.01 level
$CA_{12\ oil}$ + NBx	0.40		
$CA_{9\ oil}$ + NTx	0.43		
$CA_{9\ alc}$ + NTx	0.63		
Control	0.70		
$CA_{9\ alc}$	0.73		
$CA_{9\ oil}$	0.84		

Any two means not covered by the same line are significantly different at the level indicated.

bursectomy, or to (3) neither injection nor surgery. As above, Srbc were injected at three weeks of age, one week before serum collection and the body weight and weights of the lymphoid glands were recorded on the day of sacrifice. The effects of the various treatments on hemagglutinin synthesis are summarized in Tables II, III a-c, and in appendix tables 1.d. to 1.k.

A statistically significant reduction ($P =< .05$) in hemagglutinin titer was observed in the sera of birds treated with $CA_{9\ oil}$ + NTx, $CA_{14\ oil}$ + NTx or $CA_{9\ oil}$ + NBx. One out of seven fowls in the group treated with $CA_{9\ oil}$ alone also produced no agglutinins against Srbc, but the mean titer of the sera of this group was not significantly different from that of the control. The significantly higher ($P =< .05$) titer observed in the $CA_{10\ oil}$ + NTx group as compared to the $CA_{14\ oil}$ + NTx, $CA_{9\ oil}$ + NTx or $CA_{9\ oil}$ + NBx (Table IIIa) was totally unexpected and its significance is open to conjecture.

A reduction in thymic weight was evident in birds treated with $CA_{9\ oil}$ + NBx or oil_9 alone; the mean weight of the spleen was reduced in the $CA_{9\ oil}$ + NTx, $CA_{9\ alc}$ + NTx and $CA_{9\ oil}$ + NBx groups. Test fowls subjected to $CA_{9\ oil}$ + NTx, $CA_{9\ alc}$ + NTx, $CA_{10\ oil}$ + NTx, $CA_{10\ alc}$ + NTx or $CA_{14\ oil}$ + NTx, demonstrated only a slight reduction in their mean bursal weights; none of these differences

was statistically significant ($P =< .05$) when compared to the untreated group (Tables III b and c and appendix tables 1.e.-1.g. and 1.i.-1.k.). It was interesting to note that the administration of alcohol into the egg generally resulted in an apparent increase in hemagglutinin titer as well as in the mean weights of the thymus and the spleen; these increases were also not statistically significant ($P => .05$).

Experiment III

In this experiment the effect on the synthesis of sheep red cell agglutinins of radiation alone or in combination with thymectomy or bursectomy was investigated. In all cases, surgical removal of the lymphoid glands was performed in the neonatal period and irradiation was done at a dose of 500-800 rads, either neonatally or on the day before antigen administration at three weeks of age. The results of this investigation are illustrated in Table IV a.

Neonatal thymectomy, in combination with 700 rads gamma radiation on the second day of hatching (NTx + 700R/2d) or treatment with 700 or 800 rads gamma rays alone on the day before antigen injection, was found effective in depressing the humoral antibody response in chickens. The mean titer of the group subjected to NTx + 700R/2d was significantly lower ($P =< .05$) than that of

TABLE IV

a. The effect of gamma irradiation alone or in combination with neonatal thymectomy or neonatal bursectomy, on hemagglutinin production (Series A, Experiment III)

Treatment	Original number in group	Number of birds survived	Mean $\log_2$ HA titer (a)	Age at sacrifice (weeks)	Body weight at sacrifice (gm) mean ± S.E.	Sex ♂	Sex ♀
NTx + 700R/2d	15	12	0.75 (6)	5	176 ± 12.6	6	6
HBx + 600R/1d	6	3	0	4	169 ± 21.5	2	1
600R/2d	2	2	3.5	4	239 ± 12.5	2	0
500R/3 wk	4	4	3.0	4	239 ± 5.3	3	1
600R/3 wk	4	4	2.0	4	244 ± 12.7	1	3
700R/3 wk	4	3	2.3 (1)	4	200 ± 10	0	3
800R/3 wk	5	2	0 (2)	4	196 ± 16.9	0	2
NBx + 700R/3 wk	8	4	0	4	249 ± 24.4	2	2
Control	5	5	3.8	4	296 ± 9.2	1	4

NTx + 700R/2d = neonatal thymectomy plus 700R gamma irradiation on the second day of hatching

500R/3 wk = 500R gamma irradiation at 3 weeks of age

For explanations of other symbols and abbreviations please see legend for Table I.

TABLE IV (continued)

b. Comparison of the mean $\log_2$ hemagglutinin titers of fowls subjected to gamma irradiation with or without neonatal thymectomy (Series A, Experiment III)

Treatment	Mean $\log_2$ HA titer (a)	Significant at 0.05 level	Significant at 0.01 level
NTx + 700R/2d	0.75		
800R/3 wk	2.0		
700R/3 wk	2.3		
500R/3 wk	3.0		
600R/2d	3.5		
Control	3.8		

Any two means not covered by the same line are significantly different at the level indicated.

normal birds (Table IV b and appendix table 1.1.); fifty per cent of the chickens in this last group failed to synthesize antibody against Srbc. The mean titer of fowls subjected to 700 or 800 rads irradiation at three weeks of age (700R/3 wk; 800R/3 wk) was lower than that of the untreated control group; these reductions were, however, not statistically significant. As was expected from the results of Experiment I, NBx + 700R/2d and HBx + 600R/1d inhibited antibody synthesis to below-detectable levels. These treatments also resulted in a drastic reduction in body weight, unlike chickens treated with testosterone propionate alone (Table I). In the NBx + 700R/3 wk group, the suppressive effect of neonatal bursectomy alone was apparently enhanced by irradiation.

Experiment IV

Since Imuran (azathioprine) is known to depress immunological responses in mammals (please see Review of the Literature), an attempt was made to study the effects of this compound on hamagglutinin production in the chicken. The effect of cortisone acetate was also tested in this experiment on a single group of fowls. Various doses of Imuran were administered daily for ten days, either orally or intraperitoneally (i.p.), into randomly-selected groups of chickens; cortisone was given daily for ten days via

TABLE V. The effects of daily "imuran" and cortisone acetate treatments over a one-week period, on hemagglutinin production in chickens (Series A, Experiment IV)

Treatment (dose/kg bodywt/day)	Original number used	Number of birds survived	Mean $\log_2$ HA titer (a)	Age at sacrifice (weeks)	Bodyweight at sacrifice (gm) mean ± S.E.	Sex ♂	Sex ♀
Im. (10 mg) oral	6	6	4.7	5	343 ± 4.5	4	2
" (20 mg) "	6	6	4	5	352 ± 5.8	4	2
" (40 mg) "	6	6	5	5	329 ± 11.4	2	4
" (80 mg) "	6	6	6.8	5	317 ± 13.8	2	4
" (160 mg) "	6	6	5	5	327 ± 7.3	3	3
" (1 mg) i.p.	3	3	2.7	5	413 ± 15.2	2	1
" (3 mg) "	3	3	2.7	5	420 ± 26.4	2	1
" (5 mg) "	6	6	5	5	358 ± 7.6	4	2
" (6 mg) "	3	3	3	5	477 ± 46.8	2	1
" (8 mg) "	3	3	2	5	480 ± 34.2	3	0
" (10 mg) "	6	6	3.8	5	353 ± 6.0	2	4
" (15 mg) "	6	6	3.5	5	352 ± 13.1	0	6
CA (20 mg) "	4	4	6.5	5	292 ± 14.3	1	3
Control	8	8	2.9	5	296 ± 14.7	5	3

Im. = imuran
CA = cortisone acetate
i.p.= intraperitoneal

For explanation of other symbols and abbreviations please refer to Table I.

the i.p. route. The treatments were started three days before the injection of sheep erythrocytes and continued until the day of serum collection. All the birds were sacrificed at five weeks of age, one week after injection of Srbc; the results are summarized in Table V.

Imuran was ineffective as an inhibitor of the circulating antibody response, even with a dose as high as 160 mg per kilogram body weight per day (per os); the mean antibody titers of sera from these animals were, in fact, higher than that of the untreated control group. Cortisone acetate at a dose of 20 mg per kilogram body weight per day was also ineffective in producing impairment of the antibody response. Among the various test groups, a depression in the mean titer was obtained only in chickens treated with Imuran at a dose of 8 mg per kgm body weight.

The data obtained from this series of experiments indicated that hormonal bursectomy with either 19-nortestosterone or testosterone propionate treatment results in the abolition of hemagglutinin production against Srbc. A marked reduction in circulating antibody synthesis was obtained with NSx, $CA_{9\ oil}$ + NTx, $CA_{12\ oil}$ + NTx, $CA_{14\ oil}$ + NTx, $CA_{9\ oil}$ + NBx, NTx + 700R/2d, 700R/3 wk and 800R/3 wk treatments. The present data also revealed a general enhancement rather than a depression in the humoral response of fowls treated with azathioprine or cortisone acetate.

SERIES B

STUDIES ON THE SUPPRESSION OF THE SKIN HOMOGRAFT REACTION

The purpose of this investigation was to test the effects of various immunosuppressive techniques on homograft immune mechanisms. The treatments were essentially the same as those described in Series A and included exposure of chickens to sublethal doses of gamma radiation alone or in combination with surgical removal of the lymphoid glands, hormonal bursectomy, and cortisone treatment of the embryo with or without neonatal thymectomy. Immunosuppression was tested on skin homografts from black-feathered Lafl̀eche chickens onto M-3 White Leghorns; an autograft was also included on each individual as a control for the surgical technique.

Experiment I.a

Since the thymus and the spleen are considered to be important in the mediation of cellular immune reactions, an attempt was made in this experiment to produce an inhibition of the homograft response by the surgical removal of these glands. Two test groups were included; one group was subjected to splenectomy (Sx) alone and the other to both neonatal thymectomy and splenectomy. Sx was performed in both groups four days prior to skin transplantation;

TABLE VI. The effect of neonatal thymectomy and of neonatal splenectomy on the survival of skin homografts (Series B, Experiment I.a.)

Treatment	Original number used	Number of birds survived	Type of graft	Mean graft survival (days)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean±S.E.	Mean wt of glands at sacrifice (gm) Thymus	Spleen	Bursa	Sex ♂	Sex ♀
NTx + Sx/3 wk	6	5	auto	58	12	1028±81.3	0.10	0.57	3.83	2	3
			homo	20							
Sx/3 wk	8	4	auto	49	12	1159±49.9	6.88	0.74	4.65	2	2
			homo	22							
Control	4	3	auto	44	12	861±33.6	4.59	2.75	3.14	0	3
			homo	16							

NTx = neonatal thymectomy
Sx/3 wk = splenectomy at 3 weeks of age
auto = autograft
homo = homograft
S.E. = standard error of the mean

this time interval was intended to allow the birds sufficient time to recuperate from surgical stress. The grafts were checked weekly until the birds were sacrificed at 12 weeks of age; the body weights were recorded just before sacrifice and the lymphoid glands were weighed post mortem.

Although both neonatal thymectomy plus splenectomy and splenectomy alone prolonged the survival of homografts (Table VI), these were not significantly different ($P \geq .05$) (appendix table 2.a.) from that of the control group. The mean weights of the thymus and the bursa of the Sx-birds were greater than those in the untreated controls. Although this was strongly suggestive of an increase in lymphoid cells to compensate for the loss of the spleen, these weight gains were not statistically significant (appendix tables 2.b. and 2.c.).

Experiment I.b

In an attempt to determine the radiation dosage necessary to produce a marked impairment of the cell-mediated immunological responses, neonatal chickens were subjected to various doses of gamma rays. The birds, randomly divided into groups of two or three, were exposed to 500, 600, 700 or 800 rads gamma irradiation. Each individual was later homografted at three weeks of age;

TABLE VII. The effect of gamma irradiation on the survival of skin homografts (Series B, Experiment I.b.)

Treatment	Original number used	Number of birds survived	Type of graft	Mean graft survival (days)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.	Sex ♂	Sex ♀
500R/2d	3	2	homo	20	6	401 ± 12.1	0	2
600R/2d	3	3	homo	20	6	368 ± 34.2	2	1
700R/2d	3	2	homo	19	6	439 ± 3.6	2	0
800R/2d	2	2	homo	19	6	373 ± 50.4	1	1
Control	3	3	auto	38	6	369 ± 22.4	2	1

500R/2d = 500 rads of gamma irradiation at 2 days of age

TABLE VIII. The effect of neonatal thymectomy or neonatal bursectomy and/or gamma irradiation on the survival of skin homografts (Series B, Experiment II)

Treatment	Original number used	Number of birds survived	Type of graft	Mean graft survival (days)	Age at sacrifice (weeks)	Body wt sacrifice mean ±
NTx + 600R/2d	3	2	homo	20	7	442 ±
NTx + 700R/2d	5	2	homo	22	7	336 ± 1
Control	3	3	homo	16	7	610 ± 3
800R/3 wk	10	4	homo	24(1)	10	576 ± 3
NTx + 800R/3 wk	4	1	auto homo	44 44(1)	10	541 ±
NTx + 750R/3 wk	5	1	auto homo	54 25	11	765 ±
NTx + 700R/3 wk	5	2	auto homo	54 33(1)	11	625 ± 8
NTx + 700R/3 wk	4	2	homo	35(1)	11	868 ± 9
NTx + 700R/2d	8	4	homo	19	11	590 ± 1
NBx + 700R/3 wk	6	2	auto homo	50 19	11	984 ± 5
NBx + 700R/2d	8	2	homo	14	11	639 ± 3
NBx + 600R/2d	6	2	homo	13	11	709 ± 2
Control	3	3	auto homo	54 20	11	722 ±17

Numbers within parantheses indicate the number of homografts "accepted"

For explanations of other abbreviations please see legends for Tables VI and VII.

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Mean graft urvival (days)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.	Mean weight of glands at sacrifice (gm)			Sex	
			Thymus	Spleen	Bursa	♂	♀
20	7	442 ± 0	0.20	0.90	2.02	1	1
22	7	336 ± 10.4	0.02	0.43	0.78	1	1
16	7	610 ± 38.2	4.53	2.76	3.35	2	1
24(1)	10	576 ± 31.9	2.50	0.71	1.90	3	1
44 44(1)	10	541 ± 0	nd	nd	nd	nd	nd
54 25	11	765 ± 0	0.00	1.16	1.14	1	0
54 33(1)	11	625 ± 84	0.10	1.23	1.94	1	1
35(1)	11	868 ± 93.2	0.08	1.81	1.95	1	1
19	11	590 ± 11.6	0.12	0.83	1.83	1	3
50 19	11	984 ± 50	3.28	2.22	0.06	2	0
14	11	639 ± 30	3.51	0.83	0.00	0	2
13	11	709 ± 20	2.54	1.72	0.00	2	0
54 20	11	722 ±174.8	3.19	2.10	3.75	1	2

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TABLE IX. The effect of prenatal cortisone treatment alone or in combination with neonatal thymectomy or bursectomy, and of bursectomy on the survival of skin homografts (Series B, Experiment III)

Treatment	Original number used	Number of birds survived	Type of graft	Mean graft survival (days)	Age at sacrifice (weeks)	Body wt sacrifice mean ± S
$CA_{9\ oil}$ + NTx	4	4	homo	24	10	968 ± 31
$CA_{12\ oil}$ + NTx	4	2	auto homo	53 16	11	751 ± 30
$CA_{12\ oil}$ + NBx	8	3	auto homo	50 18	10	833 ± 58
$CA_{12\ oil}$	6	4	auto homo	53 18	11	931 ± 70
NBx	5	4	homo	24	10	919 ± 69
$HBx_{(Tp\ 12)}$	6	2	auto homo	50 19	11	1068 ± 50
Control	2	2	homo	17	10	938 ±183
Control	1	1	auto	48	10	910 ± 0

$CA_{9\ oil}$ - cortisone acetate treatment of the embryo on the 9th day of incubation

$HBx_{(Tp\ 12)}$ - hormonal bursectomy by testosterone propionate injected on the 12th day of embryonic development

For explanation of other abbreviations please refer to Tables I and II.

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an aft ival ys)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.	Mean weight of glands at sacrifice (gm)			Sex	
			Thymus	Spleen	Bursa	♂	♀
24	10	968 ± 31.3	0.02	2.37	4.84	3	1
13 .6	11	751 ± 30	0.01	2.80	1.67	2	0
10 .8	10	833 ± 58.4	4.55	2.21	0.04	1	2
13 .8	11	931 ± 70.1	5.61	5.25	4.48	1	3
24	10	919 ± 69.4	5.24	2.68	0.02	3	1
50 19	11	1068 ± 50	1.60	1.95	0.00	2	0
17	10	938 ±183.2	4.20	2.01	3.48	1	1
48	10	910 ± 0	5.44	3.35	5.03	1	0

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an autografted control group was also included in this experiment. All fowls were sacrificed three weeks after skin transplantation.

The results of this experiment are summarized in Table VII and in appendix table 2.d; they indicate that even a dose of 800 rads was not sufficient to produce a suppression of homograft immunity. The mean time of homograft rejection in the irradiated groups was not different from that in untreated control groups, which ranged between 16 and 20 days (Tables VII, VIII and IX).

Experiment II

This experiment was carried out to study the effect on the homograft reaction of irradiation alone or in combination with neonatal thymectomy (NTx) or neonatal bursectomy (NBx). The operation was performed within 48 hours of hatching. Irradiation was done when the chicks were two days old or on the day before grafting at three weeks of age. The test groups were subjected to either homograft alone, or in some cases, to both homo- and autografts. Sixty per cent of the unoperated chickens did not survive treatment with 800 rads gamma rays at three weeks of age. A high rate of mortality 75-80%) also occurred when NTx birds were exposed to 750 or 800 rads at three weeks. In each of these two groups, only one chicken survived the treatment.

The results of this experiment are illustrated in Table VIII and in appendix tables 2.e.-2.n. Heavy doses of gamma irradiation, with or without NTx, produced in some chickens a marked suppression of homograft immunity. The skin homografts which were "taken" survived as long as the corresponding autografts in one bird in each of the four test groups or for a minimum period of 30 days in the two NTx groups irradiated with 700 rads on the day before grafting at three weeks. As already mentioned, control birds retained the homografts for only 16-20 days. Grafts were "taken" only when irradiation was carried out on the day before transplantation and never when it was done in the neonatal period. NBx plus gamma radiation at any time failed to delay the rejection of skin homografts; in fact, these grafts were rejected earlier than those in the control group.

The mean weights of the spleen and the bursa of all treated groups were reduced; the thymus also showed a weight loss in all except the NBx + 700R/3 wk and NBx + 700R/2d groups. None of these differences was, however, statistically significant ($P \geq .05$) when compared to the mean weight of these glands in untreated fowls of the same age (Tables X and XI). Treatment with NTx + 700R/2d produced a significant reduction in the mean body weight of seven-week-old chickens (Table VIII; appendix tables 2.f. and 2.h.).

TABLE X. Comparison of the mean weights of the spleen and the bursa of Fabricius at 10 weeks of age, by Duncan's multiple range test (Series B, Experiments II and III)

a. Treatment	Mean weight of spleen	Significant at 0.05 level	Significant at 0.01 level
800R/3 wk	0.71		
NBx + 700R/2d	0.83		
NBx + 600R/2d	1.72		
Control (homo only)	2.01		
$CA_{12\ oil}$ + NBx	2.21		
NBx	2.68		

b. Treatment	Mean weight of bursa	Significant at 0.05 level	Significant at 0.01 level
CA_9 + NTx	4.84		
800R/ 3 wk	1.90		
Control (homografted)	3.48		

Any two means not covered by the same line are significantly different at the level indicated.

TABLE XI. Comparison of the mean weights of lymphoid glands at 11 weeks of age (Series B, Experiments II and III)

a. Treatment	Mean weight of thymus	Significant at 0.05 level
HBx	1.60	|
Control (auto + homo)	3.19	|
NBx + 700R/3 wk	3.28	|
CA_{12} oil	5.61	|

b. Treatment	Mean wt of spleen	Significant at 0.05 level	Significant at 0.01 level
NTx + 700R/3 wk(auto+homo)	1.23	|	|
NTx + 700R/3 wk(homo only)	1.81	|	|
HBx	1.95	|	|
Control (homo only)	2.10	|	|
NBx + 700R/3 wk	2.22	|	|
CA_{12} oil	5.25	|	|

c. Treatment	Mean wt of bursa	Significant at 0.05 level	Significant at 0.01 level
CA_{12} oil + NTx	1.67	|	|
NTx + 700R/2d	1.83	|	|
NTx + 750 R/3 wk	1.95	|	|
Control (auto + homo)	3.75	|	|
CA_{12} oil	4.48	|	|

Any two means not covered by the same line are significantly different at the level indicated.

Experiment III

Since in some cases cortisone treatment of the embryo followed by neonatal thymectomy or bursectomy resulted in a significant impairment of the humoral antibody response, it was thought interesting to test this treatment on the homograft immune mechanism. Thus, in the present experiment, prenatal injection of cortisone acetate on the 9th or 12th day of incubation was combined with either neonatal thymectomy or neonatal bursectomy. Control chickens were subjected to NBx, hormonal bursectomy or cortisone treatment alone. Three untreated birds were also used as controls: one of these received an autograft and each of the other two was given a homograft. The test groups were subjected to either homografts alone or to both auto- and homografts.

The results presented in Table IX indicated that, at best, only a slight delay in homograft rejection occurred in the groups subjected to NBx alone, or to cortisone acetate treatment on the ninth day of incubation followed by neonatal thymectomy ($CA_{9\ oil}$ + NTx). None of the other treatments inhibited the skin homograft response. It was interesting to note that the mean weight of the spleen in the group treated with cortisone acetate in oil on the 12th day of embryonic development was greater than in any other group, including the untreated controls. All other

treatments produced a reduction in the mean weight of the spleen. A considerable loss in thymic weight occurred as a result of hormonal bursectomy (HBx); the bursal weight was reduced in the $CA_{12\ oil}$ + NTx group. However, these variations were not statistically significant ($P \geq .05$) when compared to normal fowls of the same age (Tables X and XI).

Among the many presumably immunosuppressive techniques employed in this series of experiments, neonatal thymectomy followed by sublethal doses of gamma irradiation at three weeks of age and heavy doses of radiation alone at three weeks of age were found to produce an effective suppression of the skin homograft reaction. A slight inhibition of this immune mechanism was obtained in NTx + Sx; Sx; $CA_{9\ oil}$ + NTx; NTx + 600R/2d; NTx + 700R/2d and NBx groups.

SERIES C

STUDIES ON THE IMMUNOSUPPRESSIVE EFFECTS OF ANTILYMPHOCYTE GAMMA GLOBULINS ON FOWLS SUBJECTED TO BOTH SKIN HOMO-TRANSPLANTATION AND CIRCULATING ANTIGEN

Transplantation immunity and circulating antibody production are considered to be functionally dissociated phenomena originating in or being controlled by different cell types. In mammals and in birds, transplantation

immunity and delayed hypersensitivity reactions are closely associated with small lymphocytes - a cell type which is thought to originate primarily in the thymus. Circulating antibody, on the other hand, is believed to be synthesized by plasma cells differentiating from lymphoid cells elsewhere in the body. In birds, humoral antibody synthesis is chiefly controlled by lymphoid cells which originate in the bursa of Fabricius. Neither antibody production nor transplantation immunity, however, is solely dependent on any one type of cell, since interaction between thymus and bone marrow / bursa cells is required for immunological responses. Based on the assumption that there is some dissociation of the two mechanisms, specific suppression of each was attempted, using antiserum globulin prepared against thymus or bursa cells. Suppression of the homograft and circulating antibody responses was tested in each treated individual.

Four types of rabbit antilymphocyte gamma globulin were used in this experiment: (1) unadsorbed antithymus or antibursa gamma globulin (ATGG; ABGG) and these globulins adsorbed with (2) thymus cells (ATGG/T; ABGG/T) (3) bursa cells (ATGG/B; ABGG/B), or (4) both thymus and bursa cells (ATGG/B+T); (ABGG/B+T). The adsorptions were done to remove possible specific and cross-reacting antibodies. The experimental animals were subjected to

daily intraperitoneal injections of 0.1 ml of the specific antiserum globulin, starting on the day before transplantation and continuing until the day of sacrifice. In order to check both the circulating antibody responses and the homograft reaction, each individual was injected with 0.1 ml of 5 per cent sheep red blood cells and each also received an autograft and a homograft. Due to the relatively small amount of antiserum globulin available, the number of individuals in each experimental group was limited to three; although the birds generally withstood the treatment quite well, one chicken died in each of the ATGG/B+T and ABGG groups. The results of this experiment are illustrated in Table XII.

With the exception of ATGG/B+T, all the different types of antithymus gamma globulins used produced some suppression of the circulating antibody reaction to sheep erythrocytes; these were not, however, statistically significant ($P \geq .05$) (appendix table 3.a.). On the other hand, it is interesting to note the totally unexpected general reduction in circulating antibody synthesis in ATGG-treated birds, in contrast to its enhancement effected by ABGG treatment. This phenomenon was associated with an increase in the mean thymic weight of all ATGG-treated groups and a reduction in the weight of this organ in the ABGG groups. Of the eight types of

TABLE XII. The effects of antithymus (ATGG) and antibursal (ABGG) gamma globulin on hemagglutinin production and skin homotransplantation (Series C)

Treatment	Original number used	Number of birds survived	Mean $\log_2$ HA titer (a)	Type of graft	Mean graft survival (days)	Age at sacrifice (weeks)	Bod sa mea
ATGG	3	3	1.3(1)	auto homo	50 18	11	108
ATGG/T	3	3	2.3	auto homo	36* 16	11	93
ATGG/B	3	3	3.3	auto homo	51 30×	11	116
ATGG/B+T	3	2	5.0	auto homo	33* 18	11	112
ABGG	3	2	6.5	auto homo	35* 25	10	89
ABGG/B	3	3	8.0	auto homo	48 17	10	85
ABGG/T	3	3	7.0	auto homo	48 19	10	76
ABGG/B+T	3	3	9.7	auto homo	48 22	10	105
Control	3	3	4.0	auto homo	48 16	10	97

(a) titers expressed as reciprocals

* one bird in each of these groups "rejected" the autograft

× one bird in this group "accepted" the homograft

ATGG/T and ATGG/B = ATGG adsorbed with thymus and bursa cells respectively

Number in parenthesis represents the number of sera showing positive hemagglutinin titer

antibursal
and skin

Mean graft survival (days)	Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.	Mean weight of glands at sacrifice (gm)			Sex	
			Thymus	Spleen	Bursa	♂	♀
50 18	11	1082 ± 24.4	7.54	2.32	2.67	2	1
36* 16	11	933 ± 15.4	7.00	2.63	2.34	0	3
51 30×	11	1166 ± 71.2	6.88	2.55	3.00	2	1
33* 18	11	1124 ± 50	8.79	2.64	4.29	2	0
35* 25	10	896 ± 13.2	4.63	1.98	3.89	0	2
48 17	10	852 ± 90.5	4.74	2.14	3.04	2	1
48 19	10	765 ± 48.5	3.41	2.18	2.21	1	2
48 22	10	1051 ± 96.5	6.87	2.39	3.76	2	1
48 16	10	976 ± 0	5.89	3.17	3.65	2	1

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gamma globulins used in this series, only ATGG/B produced a marked impairment of the homograft immune response; the skin homograft was "accepted" and donor feathers grew in one out of the three chickens in this group. A slight prolongation of homograft survival was observed in the ABGG and ABGG/B+T groups.

No significant reduction ($P \geq .05$) in the mean weight of the bursa or spleen was obtained in any of the ATGG or ABGG-treated groups, as compared to untreated birds of the same age (appendix tables 3.b.-3.g.). Contrary to expectation, the mean thymic weights were generally greater in the ATGG groups and lower in the ABGG-treated batches than in normal fowls. The effect of ATGG and ABGG treatments on bursal weight was rather inconsistent. All gamma globulin treatments caused some reduction in splenic weight, whether the globulin was unadsorbed or whether it was adsorbed with homologous and/or heterologous lymphoid cells. ABGG/B and ABGG/T were the only treatments which simultaneously reduced the mean weights of the thymus, the bursa of Fabricius and the spleen.

SERIES D

THE EFFECTS OF IMMUNOSUPPRESSANTS ON SKIN HOMOTRANSPLANTATION, HEMAGGLUTININ PRODUCTION AND COCCIDIAL INFECTION

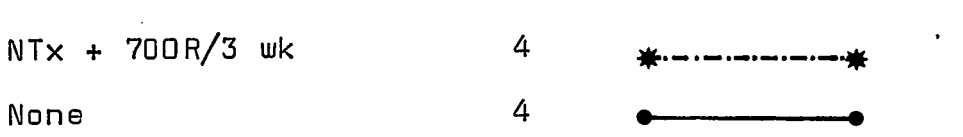
From the data presented above, it was apparent that cellular and humoral immune mechanisms in the chicken could be inhibited by various techniques. While the capacity of chickens to produce anti-sheep red cell agglutinins was abolished by hormonal bursectomy, neonatal thymectomy supplemented by gamma irradiation and anti-thymus globulin adsorbed with bursa cells permitted the establishment of skin homografts. In the present series of experiments, these methods were used to suppress the immune mechanisms involved in coccidial infections. An attempt was then made to correlate the inhibition of cell-mediated immunity or circulating antibody synthesis with the intensity of *Eimeria tenella* infection produced in the same treated birds. Thus, an alteration in the host-parasite relationship after suppression of one or another of the two immunological mechanisms would suggest which one of them plays a role in regulating the infection. The number of oöcysts eliminated in the faeces was used as the criterion for determining the intensity of infection (Davies *et al.*, 1963). An attempt was also made to study the effects of various immunosuppressive treatments on the site-specificity of coccidia.

Experiment I

The main purpose of this experiment was to determine by means of neonatal thymectomy plus 700 rads gamma irradiation at three weeks of age, a sublethal dose (800 rads) of irradiation alone at three weeks of age and hormonal bursectomy, the role of immunological mechanisms in the resistance of the host against coccidial infection. An attempt was made here to correlate the suppression of humoral or cellular immune mechanism with the intensity of infection in the same treated individual. The birds used in the present experiment were, therefore, each subjected to both skin transplantation and inoculation with sheep red blood cells (Srbc); the chickens were then infected with 100 or 1000 sporulated oöcysts of E. tenella at either three or ten weeks of age. Each treatment group had its own untreated control animals. The results of this experiment, illustrated in Figures 6-9, Table XIII and appendix tables 4.a. to 4.w., indicate that all three treatments to which the fowls were subjected had depressed the immunological state of the host.

As expected, HBx almost completely suppressed the capacity of chickens to form antibodies against sheep red blood cells, without affecting their ability to reject skin homografts. In the 800R/3 wk group and in one group of NTx + 700R/3 wk treated chickens, hemagglutinin

Figure 6. The oöcyst production curves of chickens infected with 1000 oöcysts of E. tenella at ten weeks of age (Series D, Experiment I).



NTx + 700R/3 wk = neonatal thymectomy plus 700 rads gamma irradiation at three weeks of age

Fig.6

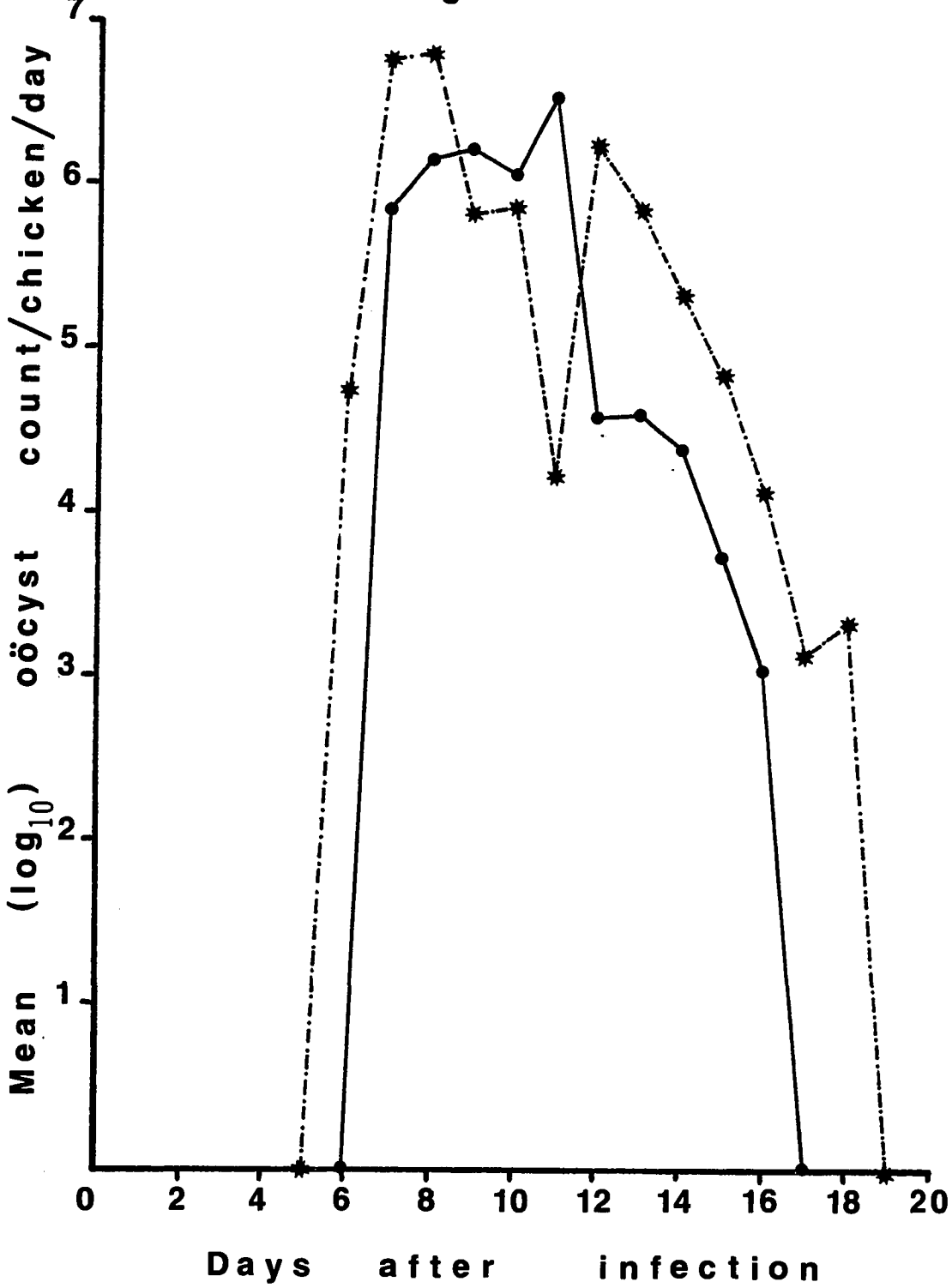
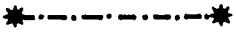

Mean (log10) oöcyst count/chicken/day
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
20
Days after infection

Figure 7. The oöcyst production curves of chickens infected with 1000 oöcysts of E. tenella at three weeks of age (Series D, Experiment I).

Legend

Treatment	No. in group	
NTx + 700R/3 wk	6	
None	3	

NTx + 700R/3 wk = neonatal thymectomy plus 700 rads gamma irradiation at three weeks of age

Fig.7

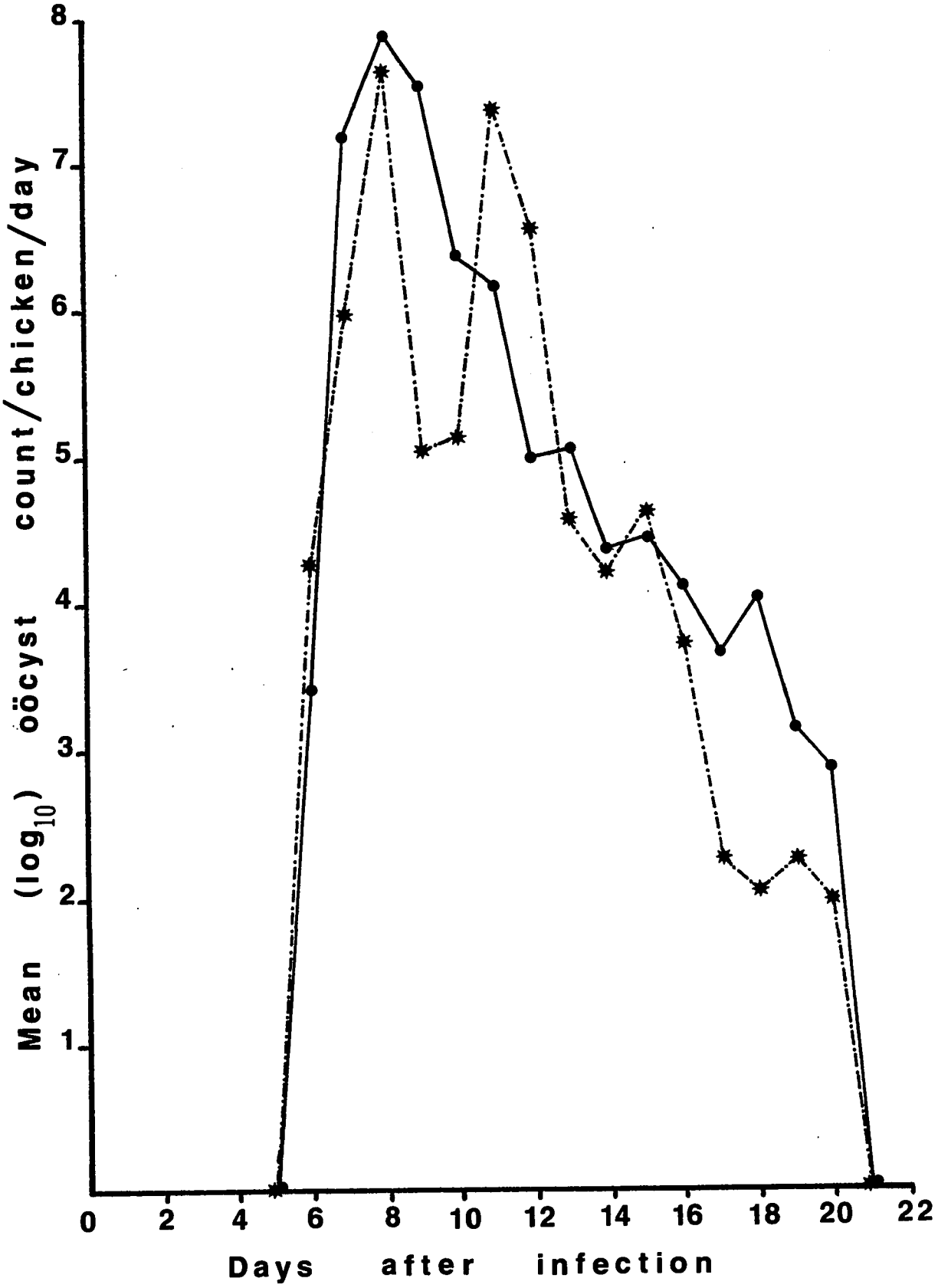
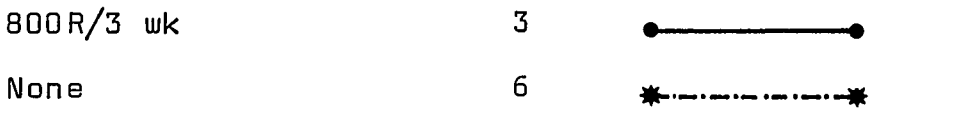

Mean (log_{10}) oöcyst count/chicken/day
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
20
22
Days after infection

Figure 8. The oöcyst production curves of fowls infected with 100 oöcysts of E. tenella (Series D, Experiment I).



800R/3 wk = 800 rads gamma irradiation at three weeks of age

Fig. 8

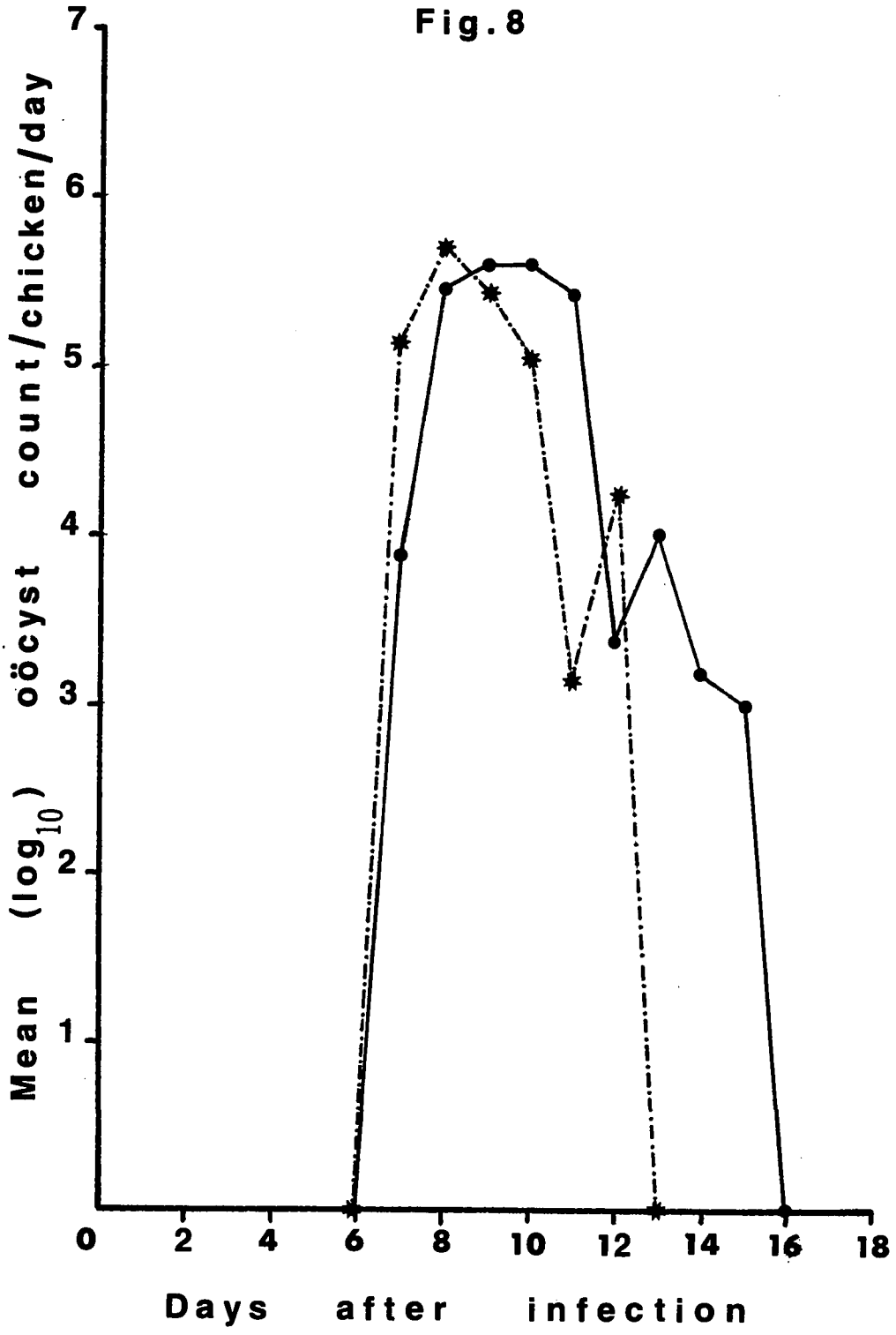
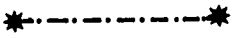

Mean (log_{10}) oöcyst count/chicken/day
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
Days after infection

Figure 9. The oöcyst production curves of chickens infected with 1000 oöcysts of E. tenella (Series D, Experiment I).

Legend

Treatment	No. in group	
HBx	5	
None	3	

HBx = hormonal bursectomy

Fig. 9

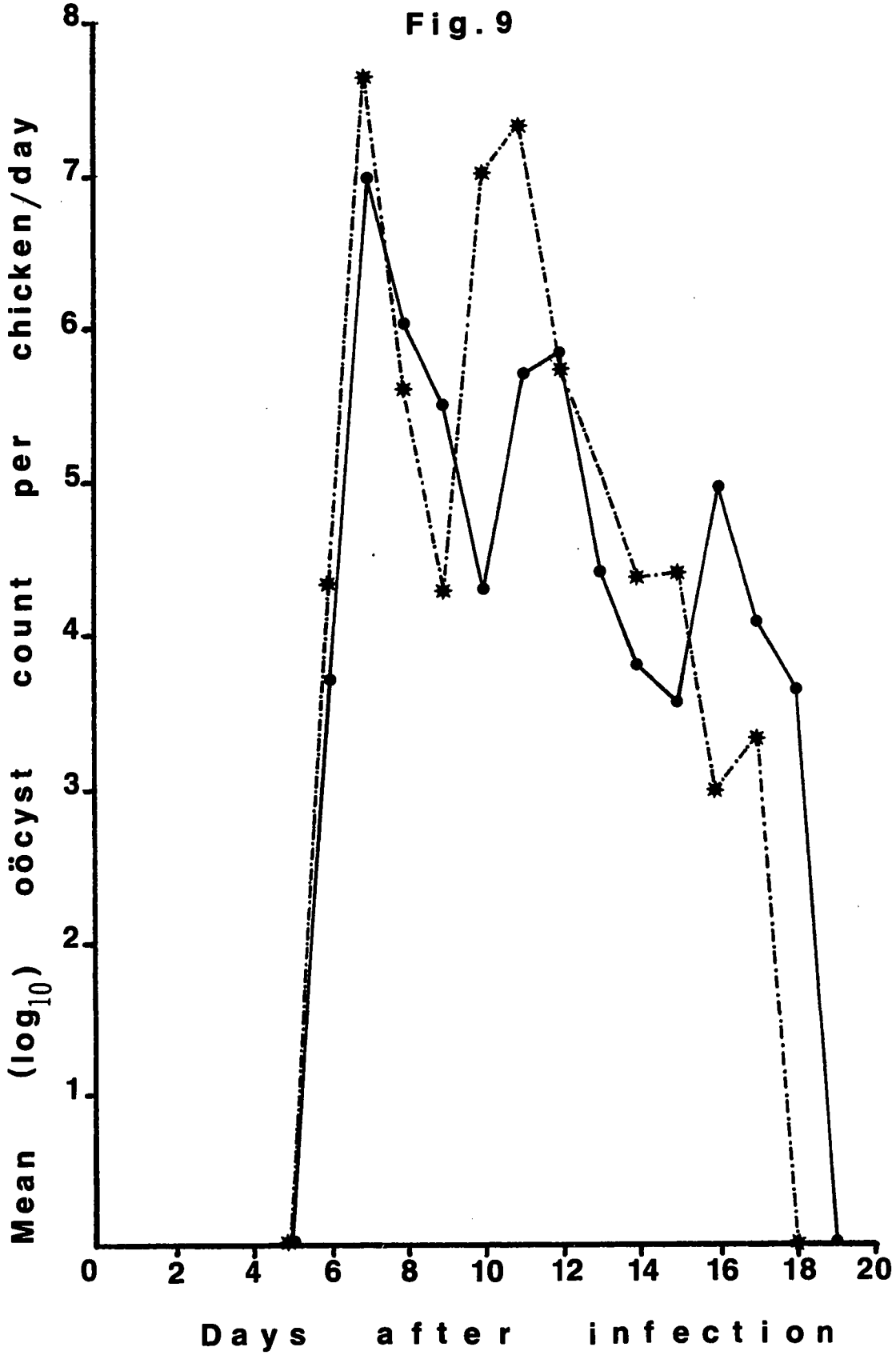

Mean (log_{10}) oöcyst count per chicken/day
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
20
Days after infection

TABLE XIII. The effects of bursectomy and of irradiation, alone or in combination with thymectomy, on transplantation immunity, hemagglutinin titer and oöcyst production of chickens infected with *E. tenella* (Series D, Experiment I)

Treatment	Original number used	Number of birds survived (a)	Mean $\log_2$ HA titer (b)	Type of graft	Mean graft survival (days)	Age sacrif (week
NTx + 700R/3 wk#	6	4	0	auto homo	72 72(4)	13
Control	6	4	3.25	auto homo	42* 18	13
NTx + 700R/3 wk	8	3	1.17	auto homo	50 23(1)	10
Control	4	2	4.20	auto homo	50 16	10
800R/3 wk	8	3	0.71	auto homo	50 17	10
Control	8	6	3.00	auto homo	50 15	10
HBx	7	4	0.33	auto homo	50 17	10
Control	4	3	5.75	auto homo	50 16	10

(a) This number does not include the 2 chickens from each group used for histology

(b) Titers expressed as reciprocals

\# These groups of chickens were infected at 10 weeks of age; all others infected at 3 weeks

* One bird in this group rejected the autograft

NTx + 700R/3 wk - neonatal thymectomy plus 700R gamma radiation at 3 weeks

HBx - hormonal bursectomy with testosterone propionate injection into the egg

Numbers in parentheses indicate the number of homografts "accepted"

e at rifice eeks)	Body wt at sacrifice (gm) mean ± S.E.	Mean wt of glands at sacrifice (gm)			Sex		Dose of oöcysts given	Total no. of oöcysts produced per bird in millions
		Thymus	Spleen	Bursa	♂	♀		
13	950 ± 80	0.22	0.68	1.69	2	2	1000	16.6
13	1293 ± 63	4.83	1.83	4.05	3	1	1000	8.2
10	686 ± 137	0.03	0.79	4.03	3	0	1000	130.3
10	886 ± 151	5.97	1.77	3.44	1	1	1000	70.9
10	686 ± 0	4.22	1.30	1.51	1	2	100	1.4
10	1057 ± 39	6.86	2.01	4.26	4	2	100	1.1
10	718 ± 25	2.57	1.21	0	2	2	1000	11.5
10	943 ± 163	4.53	1.83	4.27	1	2	1000	79.2

synthesis was significantly ($P =< .05$) inhibited (appendix tables 4.a. and 4.b.) while the humoral response was abolished in the second group of NTx + 700R-treated fowls. The homograft reaction was suppressed in five of the seven surviving birds in the two groups subjected to both neonatal thymectomy and gamma irradiation. Photographs of successful homografts showing donor feather growth on recipient (M-3) birds are shown in Figures 10-12. No impairment of the homograft reaction was evident in the three chickens that survived treatment with radiation (800R/3 wk) alone.

At the tissue level, the effect of the three different treatments seemed to have been primarily to diminish the weight of the thymus, the spleen and the bursa of Fabricius (thymectomy, of course, removed almost 95 per cent of the thymus tissue and hormonal bursectomy suppressed development of the bursa). In the 800R/3 wk group, body weight as well as the weight of the bursa and spleen were significantly ($P =< .05$) reduced (appendix tables 4.p. and 4.q.).

The number of E. tenella oöcysts produced in chickens treated with NTx + 700R/3 wk was double that of the respective controls (Table XIII); such a marked difference from the results given by the controls may indicate that this treatment can increase susceptibility

Figures 10 and 11. Donor feather growth in 9-week-old homografts of chickens subjected to neonatal thymectomy and 700 rads gamma irradiation at three weeks of age (Series D, Experiment I).

Legend

a = autograft

h = homograft

Fig. 10

Fig. 11

Figure 12. Donor feather growth in 12-week-old homografts of two chickens subjected to neonatal thymectomy and 700 rads gamma irradiation at three weeks of age (Series D, Experiment I).

Legend

a = autograft

h = homograft

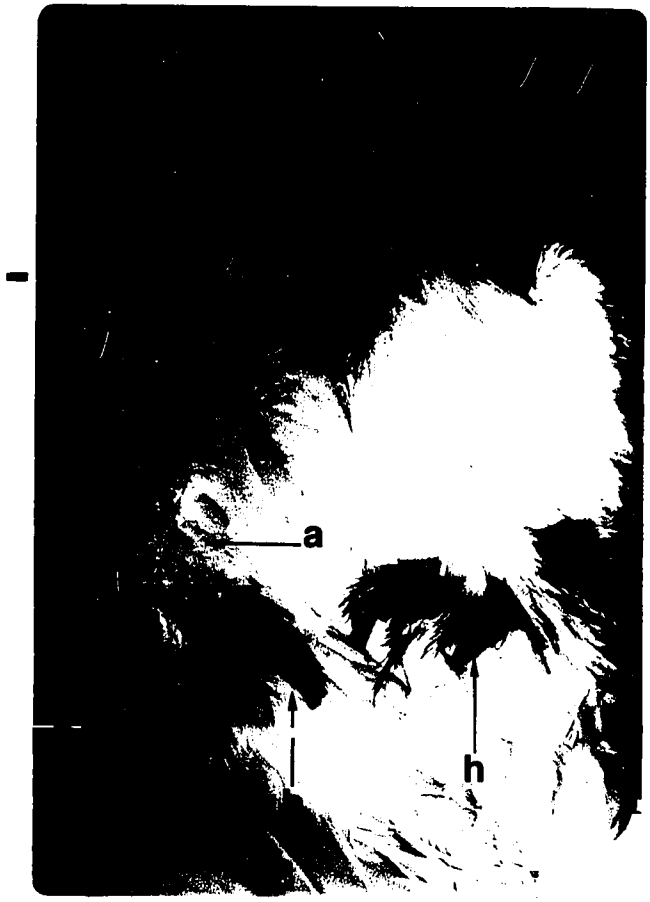


Fig. 12

to infection. This difference, however, was not found to be statistically significant ($P \geq .05$). It can be seen that in the treated group of birds which were infected at ten weeks of age, the patent period was slightly prolonged as compared to the control group (Figure 6). However, this probably resulted from additional peaks of oöcyst output caused by auto-reinfection. In those fowls infected at three weeks of age, both test and control groups demonstrated prolonged and identical patent periods (Figure 7). Gamma irradiation at 800 rads 24 hours prior to infection produced only a slight increase in oöcyst production, but this was the only treatment where the patent period of the infection was appreciably increased (Figure 8). Here also, both test and control groups showed one additional peak suggestive of auto-reinfection. Hormonal bursectomy, on the other hand, produced a depression in the total number of oöcysts eliminated, but this decrease was not statistically significant. It is interesting to note that both HBx and control groups demonstrated three simultaneous peaks of oöcyst production (Figure 9).

Experiment II

Previous experiments (Series C) on the specific inhibition of antibody formation and homograft rejection indicated that antilymphocyte gamma globulin has some effect on the immunological mechanisms of the chicken. It was therefore of interest to study the effect of anti-thymus or antibursa globulin, not only on the immunological state of the animal but also on the characteristics of E. tenella infection. In order to produce the maximum effect of the antiserum globulin (ALG) treatments, an attempt was made to induce tolerance to normal rabbit globulin (NRG) in all fowls prior to ALG treatment, as recommended by Denman and Frenkel (1967a). According to these authors, the effect of rabbit ALG is much more pronounced in animals that have previously been made tolerant to NRG; this combined treatment causes a per-sistent lymphopenia and greater destruction of lymphoid tissues, thus increasing the effectiveness of the anti-serum. The probable dosage needed to induce tolerance was determined in a preliminary experiment using human serum albumin (HSA; Nutritional Biochemicals Corporation).

Chickens were subjected to a single prenatal injection of 0.1 mg HSA dissolved in sterile distilled water into the allantoic cavity on the 12th day of embryonic development. Other chickens were given doses of 10, 20 or

40 milligrams of the antigen, either in a single intraperitoneal injection within 24 hours after hatching, or once a week for three weeks starting on the day of hatching. At three weeks of age, the prenatally-treated chicks were challenged intravenously with 1 mg HSA, and the birds treated in the postnatal period were challenged intravenously with 10 mg HSA in distilled water. The presence of anti-HSA antibody in the chicken sera was tested one week later by indirect passive hemagglutination (Stavitsky, 1954). The results of these preliminary studies (Table XIV) revealed that tolerance to HSA could be induced in 3-week-old fowls by any of the seven methods tried.

It was felt that the above data could be applied to produce tolerance to normal rabbit globulin in experimental birds. All chickens to be treated with ALG were, therefore, previously injected both during the embryonic and in the neonatal period with autologous NRG. This normal homotypic globulin was obtained before immunization from the rabbits in which the ALGs were prepared (please see Materials and Methods). The presumably tolerance-inducing treatment consisted of the injection of 0.1 ml NRG containing 7.3 mg protein/ml, into the allantioc cavity of the egg on the 12th day of incubation; this was followed by the intraperitoneal injection of an equal dose of the same NRG into

TABLE XIV. The effect of different doses of human serum albumin (HSA) on the induction of tolerance in chicks (Series D, Experiment II)

Treatment	Original number used	Number of birds survived	Mean $\log_2$ titer (a)
HSA (0.1 mg)/egg	10	5	0
" (10 mg) once	3	3	0
" (10 mg)/week	3	3	0
" (20 mg) once	3	3	0
" (20 mg)/week	3	3	0
" (40 mg) once	3	3	0
" (40 mg)/week	3	3	0
Control/challenged	3	3	5
Control/no antigen	4	4	0

(a) titers expressed as reciprocals

each chick on the first day after hatching. Antithymus (ATG) and antibursa (ABG) globulin injections were given via the intraperitoneal route at three weeks of age, in doses of 0.2 ml on alternate days, starting the day before skin transplantation at three weeks of age. The protein concentration of the ATG was approximately 4.7 mg/ml and that of the ABG preparation was approximately 14 mg/ml. Injection of sheep red blood cells (Srbc) and infection with E. tenella oöcysts were carried out within 24 hours of skin grafting. Test and control groups consisted of five chickens each; two birds in each group were used for histological examination of the digestive tract. Tissue sections were made of various regions (Materials and Methods, page 63) of the small intestine and the caeca on the fifth and seventh days after infection to determine whether any of the treatments had an influence on the site specificity of the parasite.

The effects of ATG and ABG treatments on skin homograft survival, anti-Srbc agglutinin synthesis and intensity of the parasitic infection are summarized in Table XV and in appendix tables 5.a. to 5.g.; the oöcyst production curves are shown in Figure 13. While both antiserum treatments were successful in depressing significantly ($P \leq .05$) (appendix table 5.e.) the circulating antibody response, neither treatment was able

TABLE XV. The effects of antithymus (ATG) and of antibursa globulin (ABG) on homograft response, hemagglutination (HA) titer and oöcyst production of chickens infected with E. tenella (Series D, Experiment II)

Treatment	Original number used	Number of birds survived (a)	Mean $\log_2$ HA titer (b)	Type of graft	Mean graft survival (days)	Age at sacrifice (weeks)	Body wt sacrif (gm) mean ±
ATG	8	6	2.3	auto homo	50 18	10	886 ±
ABG	8	5	2.4	auto homo	50 17	10	1029 ±
Control	6	4	4.8	auto homo	50 16	10	1057 ±

(a) The two birds used for histology are not included here

(b) Titers expressed as reciprocals

ıntibursa
.on (HA)
tenella

Age at sacrifice (weeks)	Body wt at sacrifice (gm) mean ± S.E.	Mean wt of glands at sacrifice (gm)			Sex		Dose of oöcysts given	Total no. of oöcysts produced per bird in millions
		Thymus	Spleen	Bursa	♂	♀		
10	886 ± 43	4.51	2.00	3.08	2	4	1000	2.5
10	1029 ± 24	5.00	2.10	3.90	5	0	1000	6.2
10	1057 ± 118	5.85	2.16	3.59	2	2	1000	1.1

Figure 13. The oöcyst production curves of chickens infected with 1000 oöcysts of *E. tenella* (Series D, Experiment II).

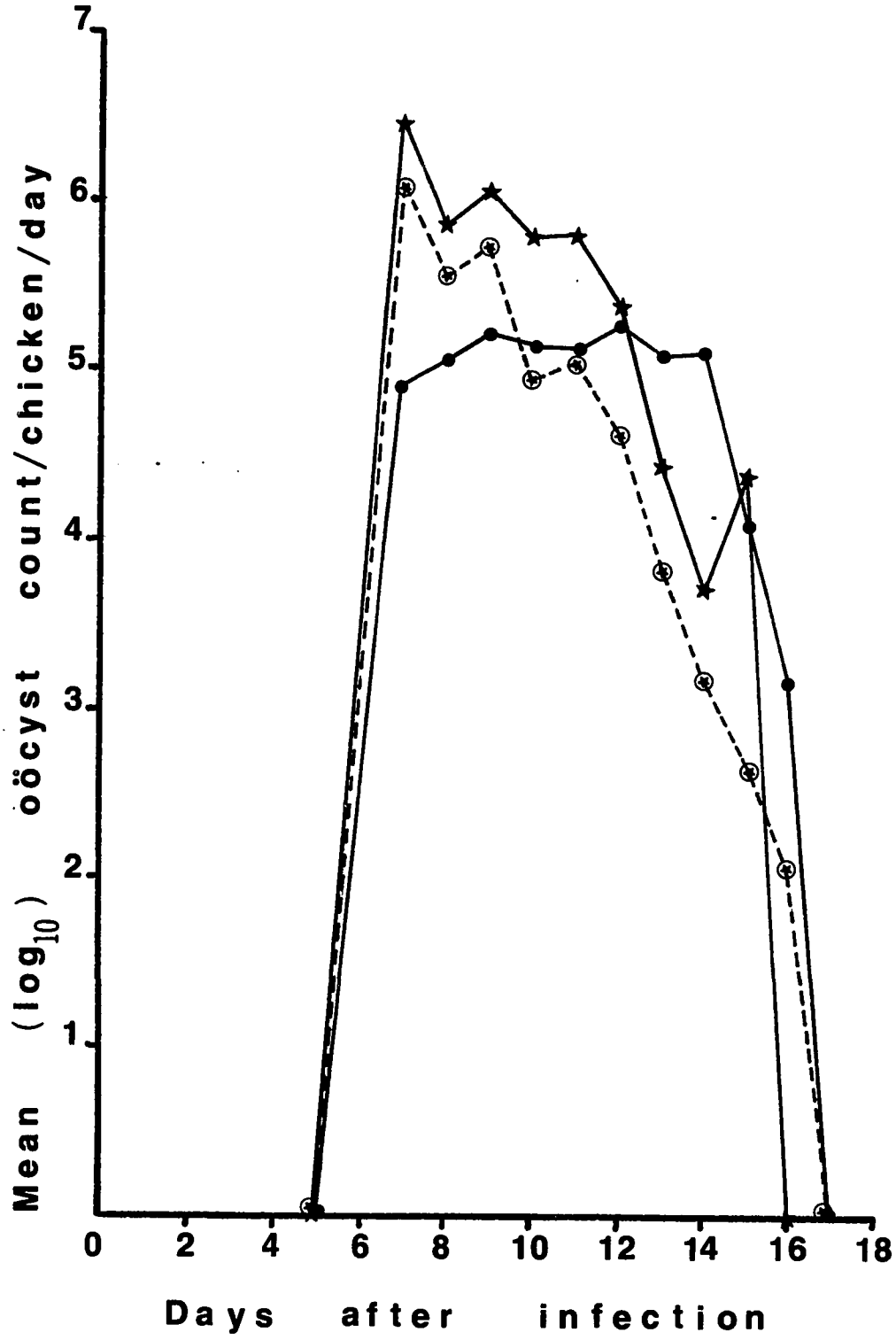
Legend

Treatment	No. in group	
ATG	6	⊕ - - - - - ⊕
ABG	7	★——★
None	4	●——●

ATG = antithymus globulin

ABG = antibursa globulin

Fig. 13


Mean (log_{10}) öocyst count/chicken/day
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
Days after infection

to produce a delay in the rejection of homografts when compared to untreated birds. There was a noticeable increase in oöcyst output in both treated groups; the number of oöcysts produced by ABG-treated chickens was approximately six times, and that produced by the ATG-treated group was twice that of the untreated controls. The oöcyst production of the treated fowls was not, however, significantly different from that of the controls. The mean weight of the thymus and bursa in the ATG-treated group was somewhat lower than it was in the control group; the mean body weight was also reduced. A slight reduction in the mean weight of the thymus was also observed in the ABG group with an unexpected increase in the mean weight of the bursa of Fabricius. Neither ATG nor ABG treatment had any effect on spleen size.

The first peak of oöcyst production occurred on the 7th day of infection in both ATG- and ABG-treated groups. In normal fowls, however, a definite peak was not established and the number of oöcysts eliminated remained somewhat constant from the 7th to the 14th day of infection. Except for this difference in the curves for the treated and control groups, the patent period of infection was almost identical in all groups. As in Experiment I, here also, it is evident that the oöcyst production curves were affected by auto-reinfection.

Histological examination of intestinal tissue revealed that the site specificity of E. tenella infection was not affected by any of the immunosuppressive treatments; the invasive stages of the parasite remained in the caeca.

It is difficult to attribute specific immunological mechanism to the host-resistance against Eimeria infection; in this aspect the present results are in agreement with those of other workers. All treatments depressed the ability of chickens to synthesize circulating antibodies against sheep red blood cells; no change in the coccidial infection could, however, be attributed specifically to this suppression. The infection was enhanced in fowls treated with ALG or NTx + 700R/3 wk and depressed in hormonally bursectomized chickens, suggesting that a thymus-dependent factor may be responsible for the regulatory effect on Eimeria infection.

The evidence presented here underlines the essentially complex nature of the immunological component in the control of coccidial infections. For example, the capacity of chickens to accept skin homografts was enhanced only in those subjected to NTx + 700R/3 wk, and yet the comparative intensity of infection in these fowls was not different from those treated with ALG in which the homograft reaction was not inhibited. Furthermore, the

infection was not affected by 800R/3 wk even though, relative to the total weight of the animal, bursal development was suppressed. It seems almost certain, therefore, that the immunological component in the mechanism which determines the natural intensity of a primary E. tenella infection in chickens is only part of a much larger system which certainly includes complicated parasite-host cell interactions.

V. DISCUSSION

In the avian system the thymus and the bursa of Fabricius are believed to possess more or less distinct immunological functions associated with cellular and humoral reactions (Aspinall et al., 1963; Szenberg and Warner, 1962). In spite of the fact that extirpation of the lymphoid glands from the neonatal animal has been employed as a reliable technique for determining the specific roles these organs play in immune responses, this method has not yet yielded conclusive results, at least as far as the avian system is concerned. Since the "central lymphoid organs" (the thymus and the bursa) become functional during the prenatal period itself (Meyer et al., 1959; Ackerman and Knouff, 1964), attempts have been directed towards suppressing their differentiation before hatching. However, such a technique has proved successful only in the case of the bursa of Fabricius. In the present investigation, neonatal removal of the thymus in combination with various other techniques, was found effective in producing a substantial impairment of thymus-controlled functions.

The present data and the results of other workers (Graetzer et al., 1963; Isaković et al., 1963) have shown that neonatal thymectomy (NTx) is ineffective as a depressant of circulating antibody response in the chicken against heterologous erythrocytes. Nonetheless, NTx has been shown to produce a complete loss of the capacity of fowls to mount a specific antibody response to some antigens and a variable loss of responsiveness to other antigens (Cooper et al., 1965). These results support the hypothesis of Aspinall et al. (1963) that the thymus cannot be completely excluded as being involved in the synthesis of circulating antibody. It is possible that the bursa cells may need to pass through the thymic environment in order to attain complete immunological maturity, as has been demonstrated in the case of bone marrow cells in mammals (Singhal and Richter, 1968; Abdou and Richter, 1969).

In contrast to the results obtained in the chicken, neonatal thymectomy in mammals produces a significant impairment of the circulating antibody response (Archer and Pierce, 1961; Miller, 1961; Martinez et al., 1962; Janković et al., 1962). However, even in mammals, the thymus is not believed to be directly involved in the production of antibodies against circulating antigens (Harris et al., 1948; Stoner and Hale, 1955 and Dixon et al., 1957).

Since in the present investigation neonatal thymectomy alone failed to diminish the circulating antibody titer to sheep erythrocytes unless supplemented by prenatal cortisone acetate treatment, it can be assumed that the hormone played some role in immunosuppression. It is likely that cortisone produced an adverse effect on the lymphoid cells as this steroid is believed to induce lymphocytolysis, as well as atrophy of the lymphatic tissue. The effect of cortisone on lymphocytes could be in the form of mitotic inhibition as well as cytoplasmic shedding (Dougherty, 1952a).

If lymphoblasts increase in thymus tissue from the seventh to the eleventh day of embryonic development as indicated by Ackerman and Knouff (1964), it could be that the impairment of antibody production after prenatal cortisone treatment and NTx obtained here was produced by the damaging effect of the corticosteroid on these blast cells. However, the fact that this treatment in the egg produced a reduction in the mean weight of the bursa and not the thymus seems to suggest that the effect of the hormone was concentrated more on the former organ. If, as suggested by Cain et al. (1969), IgM antibodies are produced by bursal cells by the eleventh day of incubation, there is a good possibility that blast cell formation and lymphoid cell multiplication are taking place on the

preceding days. One could assume, then, that injection of cortisone acetate into the egg on the ninth embryonic day could have produced lymphocytolysis as well as an inhibition in the mitotic proliferation of lymphoblasts.

The lack of an inhibitory effect when the hormone was administered on the tenth or eleventh day of incubation might suggest that cell multiplication had subsided by this time and, therefore, no interference in lymphocytopoiesis occurred. Cortisone injection on the 14th day of incubation, followed by neonatal thymectomy, also resulted in a significant impairment of the antibody response. The adverse effect of the corticosteroid on this day is more clearly due to an inhibition in the bursal tissue alone since, by this time, all lymphopoietic activity in the thymus is believed to have been completed (Ackerman and Knouff, 1964), whereas the bursa is still in the proliferative phase (Ackerman, 1962). This fact is supported by the results of Cain et al. (1969), who have indicated that differentiation of IgG-producing cells in the bursa takes place on the 15th day of embryonic development.

Since neonatal thymectomy in combination with cortisone treatment, in contrast to the latter treatment alone, was effective in inducing an inhibition of hemagglutinin reaction, it is obvious that NTx cannot be completely excluded as being responsible for the

immunosuppression obtained here. This finding may further support the hypothesis (Singhal and Richter, 1968; Abdou and Richter, 1969) that the thymic environment is necessary for the maturation of lymphoid precursor cells originating elsewhere. On the other hand, in the groups subjected to cortisone treatment alone, a recovery of lymphoid cell differentiation in the bursa could have taken place later at about the time of hatching; these cells could then undergo maturation within the intact thymus or under the influence of this gland, before reaching their final destination in secondary lymphoid sites. The variations obtained between animals in this study might be due to individual differences with regard to the time of migration of the lymphoid precursor cells.

The consistent suppression of humoral antibody reaction against foreign erythrocytes obtained in hormonally bursectomized (HBx) chickens supports the results of various other investigators (Mueller et al., 1960; Glick, 1961; Glick and Sadler, 1961; Mueller et al., 1962; Szenberg and Warner, 1962; May and Glick, 1964; Carey and Warner, 1964; St. Pierre and Ackerman, 1965; Pierce et al., 1966). The immunologic deficiency produced by this treatment can last for several months (Warner et al., 1969); these latter workers have also demonstrated that hormonal bursectomy does not equally suppress the humoral response

to antigens such as ϕX phage, dinitrophenol-conjugated bovine gamma globulin, sheep red blood cells (Srbc) and Brucella. According to Rose and Orlans (1968), antibody production against human serum albumin is also very much reduced in HBx fowls.

A dose of 2.5 mg testosterone propionate was found insufficient for complete suppression of the antibody response to sheep red blood cells. This could have been due to an incomplete inhibition of lymphoid development within the bursa by this dose, as suggested by Warner and Burnet (1961). A correlation between the amount of androgens administered into the egg and inhibition of bursal development has also been indicated by Rao et al. (1958). According to these workers, an adequate dosage of the hormone produces severe pyknosis and complete inhibition of lymph nodule formation. The time of administration of the hormone is also a determining factor in its inhibitory effect on the bursa of Fabricius; if given prior to the time of lymphoid differentiation on the 11th day of incubation, an effective suppression of the immune response can be obtained (Rao et al., 1962). It would be reasonable to assume that the immunosuppressive effect of testosterone propionate administered on the 12th day of incubation is on the immunocompetent cells produced in the bursa. Moore and Owen (1966) have suggested that the majority of bursal

cells are derived from blood-borne progenitor cells which enter the bursal primordium during embryogenesis and that testosterone treatment alters the environment in which they normally proliferate and mature. Bursa-dependent cells capable of synthesizing IgM antibodies have been indicated to differentiate in the bursa after the 11th day of embryonic development (Cain et al., 1969).

Based on the evidence of Warner et al. (1969) that recovery of the capacity to synthesize immunoglobulins in hormonally bursectomized chickens takes place by 6 to 12 weeks of age, it cannot be ruled out that testosterone propionate-inhibition of the immune response obtained in the present study was only a transient phenomenon.

In contrast to hormonal bursectomy, surgical removal of the bursa in the early neonatal period (NBx) failed to produce a significant depression in the hemagglutinin production against sheep red blood cells. Similar results were obtained by Ortega and Der (1964) when the birds were challenged with antigen at 6-10 weeks after surgery. Although several workers have demonstrated partial (Glick et al., 1956; Chang et al., 1957; Papermaster et al., 1962; Pierce et al., 1966; Cooper et al., 1966; Rose and Orlans, 1968; Stiffel et al., 1968; Cooper et al., 1969) or complete (Mueller et al., 1962; Graetzer et al., 1963; Janković and Isaković, 1966) suppression to a primary antigen injection,

a near normal antibody production to a secondary antigenic stimulus with sheep red cells was observed in NBx chickens by Janković and Isaković (1966) and Claflin et al. (1966). Furthermore, Chang et al. (1957), Cho (1963) and Graetzer et al. (1963) have shown that surgical bursectomy of older birds does not inhibit the humoral antibody reaction.

The lack of immunosuppression produced by neonatal bursectomy in the present investigation is supported by the demonstration (Cooper et al., 1966) of well-developed germinal centers and plasma cells in NBx chickens. Lymphoid cells continue to migrate from the bursa during the neonatal period (Ruth, 1960). Differences in the time of migration of immunocompetent cells from primary lymphoid sites among breeds and individuals might explain the variations observed by different investigators. The discrepancies could also be due to other factors, namely, the type, dose and time of administration of antigen.

It might be worth indicating here that the bursa of Fabricius is not believed to be solely responsible for all the immunoglobulins produced in the body (Carey and Warner, 1964; Pierce et al., 1966). Data in the literature (Thorbecke et al., 1957) favour the production of immunoglobulins in lymphoid tissues outside the bursa, especially in the spleen (Wolfe et al., 1950). If IgG antibody synthesis is exclusively under the control of the bursa as

Cain et al. (1969) have suggested, it is possible that most of these immunoglobulins produced in neonatally bursectomized birds belong to the 19S type. It might also be added that the unknown role of bone marrow cells in the immune reactions of the fowl is also worth consideration since such cells have been shown to produce precursors of immunocompetent cells in the mammalian system (Singhal and Richter, 1968; Abdou and Richter, 1969).

The spleen is considered to be the most important site in the production of antibody since plasma cell differentiation (Abramoff and Brien, 1968) and immunoglobulin synthesis (Celada and Wigzell, 1966) take place within this tissue in response to antigenic stimulation. According to White (1963, 1969), antigen is concentrated in dendritic cells within the germinal centers in the white pulp; the intense proliferation of lymphocytes in close association with these antigen-containing cells may play an important role in the "instruction" or "selection" of the antibody-producing cells. In view of these facts it was interesting that the neonatal splenectomies done during the course of this investigation produced a marked inhibition of the immune response to intravenously-injected sheep red cells. Chang et al. (1958) also obtained a reduced titer and/or slower rise in the slope of antibody production in splenectomized chickens. On the other hand,

the results of Rosenquist and Wolfe (1962) and Cooper et al. (1966) have suggested that impairment of the humoral response can be obtained in the chicken only when splenectomy is performed at the adult stage.

Since splenectomy was done by Rosenquist and Wolfe (1962) at four weeks of age at the earliest, the discrepancy between their data and that obtained in this study might be due to the difference in the age of the birds at the time of surgery. The type and dose of antigen used may also be important in determining the amount of circulating antibodies formed; the antigen used in their investigation was crystalline bovine serum albumin administered at six weeks of age or later at a dose of 40 mg per kilogram body weight. The fact that a considerable regeneration of splenic tissue was observed here in "splenectomized" chickens with positive hemagglutinin titer suggests that new precursor cells were transferred to remnants of this organ from the bursa of Fabricius and that these new cells were responsible for the immune reaction. Such a view has been previously expressed by Papermaster and Good (1962), Graetzer et al. (1963) and Woods and Linna (1965). The complete suppression of the antibody titer against Srbc observed here in some of the splenectomized chickens could be due to a lack of sufficient immunocompetent cells for responding to

antigenic stimulation thereby supporting the concept (White, 1963, 1969) that the splenic environment is essential for the synthesis of immunoglobulins in the chicken.

There is sufficient evidence in the literature to indicate a direct role for mammalian spleen cells in the production of circulating antibodies (Eveland, 1964; Nakano and Braun, 1965; de Petris and Karlsbad, 1965; Hanna et al., 1966). Pyronin-staining lymphoblasts appear in the white pulp of the spleen about 24 hours after antigenic stimulation and by 48 hours these cells show structural features characteristic of cells involved in active protein synthesis; this stage is followed by the appearance of plasma cells. A highly organized rough-surfaced endoplasmic reticulum develops in a large number of these plasma cells three days after antigenic stimulation followed by the appearance of antibody in the serum (Hanna et al., 1966).

In addition to the extirpation of lymphoid organs, exposure of animals to heavy doses of X-irradiation has been extensively used as an immunosuppressive technique. Lymphoid tissue is particularly sensitive to this treatment because its high rate of division is susceptible to mitotic inhibition by "hard" penetrating rays such as X- and gamma rays.

In the present study, doses of 700 and 800 rads gamma rays before antigen injection produced marked impairment of the immune response; similar results were also obtained by Dixon et al. (1952), Makinodan et al. (1962), Taliaferro et al. (1964) and Williams (1966). Radiation damage has been indicated to be chiefly centered on the proliferative phase of lymphoid cells that occurs during the second part of the latent period (Taliaferro et al., 1964). However, since this phase does not take place before antigenic stimulation, it is difficult to associate the effect of pre-immunization radiation with the proliferative activity of immunocompetent cells. A more acceptable explanation would be an adverse effect of irradiation on the genetic information of the as-yet uncommitted lymphoid stem cells or of the lymphocytes themselves, as suggested by Murray (1948). He demonstrated that irradiation of rabbits with doses of 400 to 800r resulted in the abnormal clumping of chromatin, leading to the death and elimination of a large number of lymphocytes from the spleen. Circulating lymphocytes are also affected by radiation (Taliaferro et al., 1964). In addition, Williams (1966) has indicated the damaging effects of radiation on the antigen-trapping mechanism of the immunological response. Since lymphoid cells are also involved in other functions such as intermediary metabolism

and in the storage of nutritive materials (Kelsall and Crabb, 1958), the interference of radiation in immune reactions might also be the indirect result of a derangement of normal metabolic activities. The processes involved in 7S antibody formation are believed to be more radiosensitive than those of 19S antibody (Svehag and Mandel, 1964; Sahiar and Schwartz, 1965; Nettesheim et al., 1969); it can be assumed, therefore, that complete inhibition of the hemagglutinin titer in heavily irradiated chickens in these experiments probably indicated the absence of both 7S and 19S antibodies, whereas in birds subjected to lower doses of gamma rays only the 7S antibody was suppressed.

Considering the damaging effects produced on the lymphocyte population by heavy doses of irradiation, it was thought worthwhile to supplement neonatal thymectomy with whole body irradiation, with a view to eliminating all thymus-dependent cells, especially those which had already migrated to secondary sites during prenatal development. The results showed that 50 per cent of the chickens subjected to surgical thymectomy followed in the neonatal period by irradiation at a dose of 700 rads, failed to produce detectable amounts of circulating antibodies to Srbc at three weeks of age. Since a period of one to two months is necessary for recovery from heavy doses of

radiation (Taliaferro and Taliaferro, 1964), the immunosuppression produced in these birds was probably due to the adverse effect on the lymphoid population as a whole rather than on the thymus-dependent system alone. It can be concluded, therefore, that the impairment in this test group was chiefly due to an irradiation-induced deficiency in the bursa-dependent tissue since neonatal thymectomy alone does not efficiently depress the antibody response to sheep erythrocytes. This view is supported by the complete suppression of hemagglutinin titer in the neonatally bursectomized-irradiated chickens.

The extensive use of purine analogues and corticosteroids as immunosuppressive agents in the recent past prompted the present studies on the effects of azathioprine (Imuran) and cortisone acetate on the circulating antibody response. The results were, however, not encouraging; both compounds produced an enhancement of the hemagglutinin response! Data on the effects of Imuran on circulating antibody synthesis is scanty. Nevertheless, the effects of this compound can be compared to those of its analogue, 6-mercaptopurine (6-MP), which has been used extensively as an inhibitor of the humoral antibody response since Elion et al. (1961) have indicated that in mammals, Imuran is converted to 6-MP and its oxidation product, 6-thiouric acid. In addition, both these compounds undergo similar

metabolic processes (Elion et al., 1961) and both have been shown to block the pathway of purine synthesis at identical sites (Berenbaum, 1965).

Enhancement of circulating antibody production by 6-MP has been demonstrated in the rabbit by Chanmougan and Schwartz (1966). These investigators postulated that such enhancement was caused by the release of nucleic acids from cells killed or injured by the drug and that lymphocytes which incorporate these nucleic acids were transformed into special types of lymphoid cells (hemocytoblasts) which lack immunological memory. Since a small dose of antigen is capable of stimulating all the hemocytoblasts representing a given clone, large amounts of antibody were released rapidly into the circulation. There is also the possibility that the enhanced hemagglutinin response induced by Imuran represented only the 19S type of immunoglobulins; this globulin has been indicated to be less sensitive to 6-MP than the 7S type (Sahiar and Schwartz, 1965). Such a view is supported by the results of Borel et al. (1965) who found that 6-MP treatment in mice and rabbits resulted in a delay and reduction of 7S immunoglobulin formation and a prolongation of 19S antibody synthesis.

The lack of suppression obtained by cortisone injection in three-week-old chickens in this study is

supported by the results of Glick (1967) who showed that neonatal treatment of chickens with this hormone failed to inhibit the hemagglutinin reaction at six weeks of age. Nonetheless, it is quite possible that, in the latter case, a recovery of the lymphoid cells could have taken place during the interval of six weeks between the time of treatment and antigen administration. Dameshek et al. (1951) were also unable to produce an impairment of the agglutinin response in patients who underwent "short term" cortisone therapy. Dougherty (1952a), however, obtained successful inhibition of circulating antibody production in mammals by cortisone treatment. Neonatal treatment of chicks with heavy doses of this hormone was also shown to be effective in depressing the precipitin titer against bovine serum albumin administered at six weeks of age (Glick, 1967). The lack of antibody suppression obtained in the present results could be due to factors such as insufficient dose and/or an inappropriate time of the treatment in relation to sensitization with the antigen.

Several of the techniques described above were also used in an attempt to suppress the immunological mechanisms involved in homograft rejection. Since an important role in reactions associated with cytophilic antibodies has been attributed to the thymus, most of these studies were aimed at curtailing the influence of this organ in

transplantation immunity. Neonatal thymectomy has been the method most commonly used for determining the immunological role of this organ. In the present investigation, this technique was supplemented by other treatments since NTx alone has been shown to be incapable of suppressing the homograft reaction in chickens (Aspinall et al., 1963).

When the fowls were subjected to both NTx and splenectomy (Sx), only a slight delay in the rejection of skin grafts was observed. Szenberg and Warner (1962) postulated that thymus-dependent cells in the spleen and other secondary lymphoid organs are responsible for cellular immune mechanisms so that the eventual rejection of the skin homograft might have been effected by the splenic tissue which had regenerated in all Sx birds. This tissue could have originated from the remnants of the spleen and/or the thymus which escaped removal during the operation; in addition, the spleen has the ability to respond to antigen in the absence of the thymus (Cooper et al., 1965). The small lymphocytes in circulation might also have contributed to the immune reaction; a role of these cells in graft rejection has been indicated by Gowans et al. (1962) and by Berrian and Brent (1958). Although neonatal thymectomy might have caused a reduction in the peripheral lymphocyte pool, as Miller (1961) has shown, it is likely that by three weeks of age a recovery

of this pool was accomplished by contributions from other lymphoid organs. Since the spleen was removed only four days before grafting, a residual population of lymphocytes could be expected in the circulation at this time. The similarity of results obtained after NTx + Sx, or Sx treatment, once again supports the concept that by three weeks of age the spleen has become an important lymphoid site capable of responding to antigenic stimulation in the absence of the thymus. Such a view also finds support in the works of Ruth (1960), Fichtelius (1960), Miller (1961), Waksman et al. (1962) and Warner (1965) who have suggested that, in the mammalian system, precursors of immunologically competent cells move out from the thymus to colonize the spleen and other lymphoid areas during the first few weeks of life. It will be recalled that an appreciable increase occurred in the mean weights of both thymus and bursa in the splenectomized group suggesting a hypertrophic reaction of these organs in response to splenic deficiency. The slight increase in bursal weight in the NTx + Sx group, plus the evidence of eventual rejection of the homograft, might support the view expressed by Aspinall et al. (1963) that the bursa does play a minor role in the rejection of skin grafts.

The failure of cortisone injection into the embryo in combination with neonatal thymectomy, to inhibit the

immune responses involved in skin homograft reaction in this investigation is in general agreement with the results of Aspinall and Meyer (1964). These investigators were, however, able to produce some delay in homograft rejection with cortisone treatment and thymectomy during the neonatal period. Neonatal administration of cortisone alone was also found effective in suppressing the cell-mediated immune response (Meyer et al., 1964).

Irradiation alone is not particularly effective in suppressing the cellular antibody response (Toolan, 1953). In the present experiments, neonatal exposure of chickens to radiation failed to induce acceptance of skin homografts. The lack of suppressive effect of this treatment on homograft rejection, even with a dose as high as 800 rads, might indicate that either the cells involved in transplantation immunity can completely recover from the adverse effects of irradiation during the three-week interval between treatment and grafting or that the cells involved in this immune reaction are less radiosensitive than those associated with the humoral response. The former view is supported by the results of Makinodan et al. (1962), who found that recovery from the damaging effects of radiation starts at one week after exposure to X-rays. The latter assumption may also be of significance since Uhr and Scharff (1959) have indicated that the capacity to develop

a delayed type of hypersensitivity reaction may persist in X-irradiated guinea pigs, even when the ability to form circulating antibody is curtailed. It has been known for some time (Jacobson, 1954) that lymphocytes which are actively involved in antibody synthesis are more radiosensitive than other cells.

On the other hand, when irradiation and thymectomy were both done in the neonatal period in this investigation, a slight prolongation of graft survival occurred. A much greater delay in homograft rejection was obtained by Cooper et al. (1966) in chickens subjected to similar treatments. These data confirm the view that the neonatal thymus plays some role in homograft immunity as postulated by Warner and Szenberg (1962), Aspinall et al. (1963), Aspinall and Meyer (1964) and Warner and Szenberg (1964). According to Janković and Isaković (1964) and Cooper et al. (1966), the thymus-dependent system which is responsible for cellular immunity is represented in the spleen, the gut and other secondary lymphoid organs. The damage produced in these sites by radiation is, apparently, centered on a large proportion of the lymphoid precursor cells already migrated there from the primary tissues. The eventual rejection of the grafted skin in this study by neonatally thymectomized-irradiated birds could have been effected by the cells which survived and which

multiplied during the three weeks between treatment and grafting, and later differentiated into fully competent cells in response to stimulus by transplantation antigens.

More favorable results were obtained here when neonatal thymectomy was followed by gamma radiation on the day before skin transplantation. The higher level of immunosuppression produced after neonatal thymectomy by a dose of 700 rads than by 750 rads could be explained by the more severe effect of the higher dose on the body since it is close to the lethal level (Taliaferro and Taliaferro, 1964). In contrast to these results, birds which were neonatally bursectomized and then irradiated 24 hours before skin grafting rejected the homografts in normal time, thereby supporting the view of Janković et al. (1963) that the bursa may not play a significant role in the homograft reaction.

It is likely that irradiation produces a deficiency in the lymphocyte population in both the peripheral blood and the lymphoid organs. Furthermore, Dempster et al. (1950) have suggested that only the inductive phase of the heterograft reaction is radiosensitive; irradiation at any other phase of the immune response is generally without effect. Irradiation affects the digestive efficiency of macrophages (Jaroslow, 1959) and the antigen-trapping mechanism of lymphoid follicles within the lymph nodes of the rat (Williams, 1966). The mean weights of the bursa

and the spleen of all irradiated groups in the present experiments were noticeably reduced in relation to those of normal birds. An inverse correlation between the dose of radiation and the mean weights of these lymphoid glands was also apparent. Exposure of unoperated birds to a sub-lethal dose (800 rads) of gamma rays at three weeks of age produces some inhibitory effect on the immunological response since it resulted in a significant impairment of the humoral antibody reaction in one out of the four chickens tested. Although the thymic tissue is considered to be highly radiosensitive because of its relatively high mitotic rate (Miller and Osoba, 1967), in the present study treatment with radiation alone was found to be less effective on the homograft response than when it was preceded by NTx.

As immunosuppressive agents, heterologous anti-lymphocyte (ALS) and antithymocyte (ATS) sera produce drastic changes in the activity of immunocompetent cells (Berenbaum, 1967; Reithmuller et al., 1968; Barth et al., 1968). Various theories have been proposed to explain the mechanism by which the action of these sera is exerted on immunocompetent cells. Recently, Barth et al. (1969) have postulated that this effect may be due to the direct action of antilymphocyte gamma globulin on antigen-sensitive macrophages which are involved in the processing of antigen in the early stages of the immunological response.

In the present experiments (Series C), treatment with antithymus gamma globulin (ATGG) produced a partial inhibition of the hemagglutinin reaction in all groups except in that treated with ATGG adsorbed with both thymus and bursa cells (ATGG/B+T); in this group of birds there was an enhancement of antibody formation against sheep erythrocytes. The greatest depression in agglutinin titer was observed in the group treated with unadsorbed ATGG. The present results are in accord with those obtained by Berenbaum (1967) in mammals subjected to antithymus serum treatment. Jankovíc et al. (1970) inhibited antibody production in chickens against bovine gamma globulin (BGG) with rabbit antithymus (ATG) and antibursa (ABG) globulins. Antibody production in the present study was suppressed to a lesser extent in chickens treated with ATGG which had been adsorbed with thymus cells (ATGG/T). Asakuma and Reif (1968) also found that the potency of ATS is lowered when adsorbed with thymus cells. When antithymus globulin was adsorbed with bursa cells (ATGG/B), the capacity of the antiserum to inhibit antibody synthesis was almost abolished. This result suggests that there are bursa-dependent cells in the thymus and that the inhibitory effect of other serum treatments on antibody formation can be ascribed to the action of the globulins on these cells. As suggested above, there is the possibility that bursa-dependent cells may need to enter the thymic environment before reaching the secondary lymphoid sites to undergo proliferation and

eventual differentiation into antibody-synthesizing cells. The results presented here further suggest that there may be a physical association between both cell types within the thymus. The concomitant decrease in bursal weight in all but the ATGG/B+T group adds further evidence in support of the view that bursa-derived cells may be present in the thymus.

The increase in mean weight of the thymus after anti-thymus gamma globulin treatments is in agreement with the report of Marshall and Knight (1969) and could be a manifestation of increased proliferative activity in the thymus to compensate for the deleterious effect of the antiserum on the gland itself as well as on the peripheral lymphocytes. ATG and ABG treatments can produce lymphocytopenia in the chicken but do not influence the lymphoid structure of the thymus (Janković et al., 1970). ATS has been shown to produce a more persistent lymphopenia than antiserum prepared against lymphocytes (Nagaya and Sieker, 1965, 1969); these investigators have also indicated that the greater immunosuppressive activity of ATS, as compared to ALS, is due to the ability of the former serum to deplete thymic lymphocytes in addition to those in circulation.

It could be hypothesized that the reduction in splenic weight resulting from all ATGG-treatments used in this study was caused by an increased mobilization of lymphocytes from this organ to compensate for a generalized lymphopenia. Such an idea finds support in the demonstration (Barth et al.,

1969; Janković et al., 1970) of lymphoid depletion in the spleens of ATS-treated animals. A similar reduction in splenic weight (Turk and Willoughby, 1967) and widespread death of lymphocytes within this organ (Woodruff and Anderson, 1964) have also been observed in ALS-treated animals. In addition, the number of small lymphocytes is reduced in animals after treatment with antithymus serum adsorbed with spleen cells (Lueker and Tribble, 1969).

The skin homograft reaction was suppressed in this study only by antithymus serum adsorbed with bursa cells; this fact seems to indicate that there is an interaction between the two "primary" lymphoid organs (thymus and bursa), and that the role of the thymus in graft rejection may partially depend on bursa-derived cells. The possible interaction between bursa and thymus cells in the antibody response has been noted above. Adsorption of ATGG with bursa cells might have functionally "unmasked" more thymus-specific antibody sites in the serum; the occurrence of specific antigens in the thymocytes which are absent from other lymphoid cells, has been demonstrated by Potworowsky and Nairn (1967). If there is in fact an exchange of cells between the bursa of Fabricius and the thymus, it can be assumed that unadsorbed antithymus serum might also affect the bursa-derived cellular elements within the thymic environment, since the thymus cell suspension used to prepare the ATGG seems also to have contained bursa-specific antigens. Such bursa-specific antigens have been demonstrated by Forget et al. (1970).

Tucker (1968) was able to induce a delay in skin homograft rejection in chickens by antilymphocyte serum treatment. This is supported by the demonstration (Denman and Frenkel, 1968a) that ALS produces a damaging effect on the peripheral lymphocytes which are believed to be involved in the homograft response (Gowans and McGregor, 1965). Kinne and Simmons (1967) also found that there was a close correlation between lymphopenia and homograft retention in ALS-treated mice and according to Turk and Willoughby (1967), the effect of ATS in the mammalian system is specifically directed against the thymus-dependent areas of lymph node tissues concerned with cell-mediated immune responses. Inhibition of immunological reactions by ALS might be effected by the blocking of the specific combining sites and/or the recognition units of sensitized lymphocytes (Levey and Medawar, 1966). These investigators have indicated, however, that the effects of ALS-treatment on homograft immunity cannot be entirely accounted for by lymphocytes alone, since homografts can be maintained by continuous antiserum treatment despite a rise of peripheral lymphocytes to normal levels. Their results seem to support the "sterile activation theory" which proposes that anti-lymphocytic antibody stimulates the production of normal numbers of lymphocytes but that these newly-formed cells lack immunological potential (James, 1967).

Because ATS is known to be more effective than ALS in inducing an impairment of thymic functions, a similar study was conducted with sera prepared against bursa cells to determine what effect they would have on the synthesis of antibodies. Although it was expected a priori that antibursa gamma globulin (ABGG) would suppress the bursa-dependent function of circulating antibody reaction, this treatment produced, paradoxically, an enhancement of the hemagglutinin titer. This phenomenon could have been due to an increase in the activity of the spleen, manifested by a general increase in its weight in all ABGG-treated groups. The present results also imply that since the ABGG was prepared against the almost exclusively large and immature bursal lymphocytes (Peterson and Good, 1965), the antibodies in the serum could have acted specifically on these and not on an immunocompetent population of small lymphocytes. Those immature lymphocytes which escaped the effect of the antiserum could have undergone rapid compensatory proliferation in response to subsequent antigenic stimulation with sheep red cells; this could have yielded sufficient numbers of immunocompetent cells to produce an enhancement of the agglutinin titer. Such a hypothesis finds support in the demonstration of increased numbers of plasma cells in the lymph nodes of rats treated with ALS (Denman and Frenkel, 1968b). In addition, Tyler et al.

(1969) found that antilymphocyte serum stimulates some of the small lymphocytes in circulation and transforms them into large blast cells. A mitogenic action of antileucocyte immune serum on the peripheral lymphocytes has also been proposed (Gräsbeck et al., 1963). The highest increases in circulating antibody formation were observed in the groups treated with ABGG/B and ABGG/B+T; this enhancement was most probably due to the stimulation of bursal tissue.

Once the effect of the various immunosuppressive treatments on the cellular and humoral responses of the chicken had been established, some of the most successful of these techniques were applied to infection with Eimeria tenella; the main purpose of this investigation was to determine whether immediate or delayed hypersensitivity mechanisms play any role in the control of Eimeria infections in the fowl. In spite of the studies conducted in the past by other workers, the immune mechanisms involved in coccidial infection are still not clearly understood.

Among the different techniques employed in this study, all except 800 rads gamma irradiation at three weeks of age and hormonal bursectomy produced a noticeable increase in oöcyst production. The effect of antithymus globulin (ATG) on the infection was not as great as that

caused by antibursa globulin (ABG). Euzeby et al. (1969) also demonstrated that ATS treatment produced only a slight depression in the immune response of chickens against E. tenella infection. Similar results were obtained by Rose (1968) in her studies using antilymphocyte serum. Rose and Long (1970) and Rouse and Burns (1971) found that neonatal thymectomy had little effect on infection with species of Eimeria. The fact that the number of oöcysts eliminated by ATG-treated chickens used in the present study was approximately twice that of the control birds implies, however, that the thymus or a thymus-controlled system may play some role in host resistance. Nonetheless, it is possible that the dosage used here was insufficient since Gray et al. (1964) have shown that a daily dose of 0.25 ml of ALS was necessary in the mouse to produce a fall in the total lymphocyte count in circulation to 40 per cent of the normal level; this dose was very much higher than that used here when the relative weights of the two different animals are taken into consideration.

The lack of a more pronounced effect of ATG in this study might also suggest that sufficient numbers of immunocompetent cells were present in secondary lymphoid sites such as the spleen, the Peyer's patches and the tonsilla caecalis (Janković and Mitrović, 1967; Janković, 1968) to

compensate for the damaging effects of the antiserum globulin. It is well known that the adverse effects of ALS is chiefly centered on the lymphocytes in circulation. The effect of ATG on the thymus gland itself was manifested by a reduction in mean weight. This could be due to a compensatory mobilization of lymphocytes into the circulation in response to the detrimental effects produced by the immunosuppressive agent.

The importance of the integrity of the bursa, or of a bursa-dependent system in the synthesis of circulating antibody was once again evident in the results obtained (Series D) in chickens treated with antibursa globulin. Although antibody formation against Srbc was not abolished in all individuals, a consistent reduction in titer was observed. Associated with this was an apparent enhancement of susceptibility to infection in the treated group, manifested by an increase in the total number of oöcysts produced. It is clear from the curve that most of the increase in oöcyst production in the test group occurred during the first part of the patent period. Euzeby et al. (1969) also found that antibursa serum had a more pronounced suppressive effect on coccidial immunity than that produced by ATS treatment. The importance of the bursa of Fabricius in host resistance to coccidial infection is also suggested by the work of Challey (1962). Rose and

Long (1970) found that twice as many E. brunetti oöcysts were formed in neonatally bursectomized-irradiated chickens as in controls which had only been irradiated. These data imply that circulating antibodies play some role in coccidial immunity. Such a view has been expressed by Itagaki and Tsubokura (1955), Horton-Smith et al. (1961), Pierce et al. (1962), Long et al. (1963) and Herlich (1965). Protection against the parasitic infection can be achieved by passive transfer of immune serum (Rose, 1971). The lack of effect of hormonal bursectomy in increasing the host's susceptibility to Eimeria infection in the present study is in accord with the results of Long and Pierce (1963), Sadler and Edgar (1963), Pierce and Long (1965) and Rouse and Burns (1971).

Considering the above data, it is possible that the bursa of Fabricius is involved in two types of reaction: (1) circulating antibody production and (2) induction of host resistance against coccidial infection, both of which may be functionally distinct. This conclusion could, then, lead to the hypothesis that the response of the host against this caecal parasite may be a local phenomenon regulated by the central lymphoid organs. If the action is controlled at the local level, removal of the central organs may be without effect whereas the more wide-ranging ALG or ALS treatment may exert some action; the role of

the spleen in this process would be less important (Rose, 1968) in spite of its significance in humoral antibody reaction.

The local production of antibodies by lymphatic tissues associated with the gut was demonstrated by Farr et al. (1960), Farr and Dickinson (1961), Janković and Mitrović (1967) and Remington (1967). This particular reaction may be associated with heterophil polymorphonuclear cells, pyroninophilic cells and "globule leucocytes" that infiltrate the sites of infection (Pierce et al., 1962). The studies of Kent (1952) have indicated that the globule leucocytes usually found in the gut wall of the sheep may originate from small lymphocytes in response to the diffusion of antigens into the mucous membrane; this observation is especially interesting in view of the demonstration (Dobson, 1966) of globulins in the cytoplasm of these cells. A similar function could be proposed for the globule leucocytes that infiltrate the mucosal tissues of birds (Horton-Smith and Long, 1963) during coccidial infection. According to Becker (1934), immunity to E. miyairi infection in the rat may be a purely local phenomenon associated with an immune principle which is capable of spreading from cell to cell. Rose (1963), on the other hand, suggested that the immune response in coccidiosis might be controlled by a combination of local and humoral factors.

Treatment of fowls with neonatal thymectomy in combination with 700 rads gamma irradiation on the day before infection resulted in an increased oöcyst production by E. tenella. The fact that this treatment induced a concomitant depression of skin homograft immunity in the infected group suggests a possible role for cell-mediated immune reactions in the host response to coccidial infection. The importance of cellular immunity in intracellular parasitism has also been indicated by Gray and Cheers (1969). The present results are in agreement with those of Rouse and Burns (1971) who found that thymusless birds were slightly more susceptible to a primary infection with E. tenella. On the other hand, Rose and Long (1970) were unable to produce an inhibition of immunity to E. maxima by NTx in conjunction with irradiation. However, irradiation was carried out by these investigators during the neonatal period and infection followed at three weeks of age or later; it is therefore possible that these birds had sufficient time to recover from the adverse effects of the treatment on their immune mechanism. Furthermore, the work done in the present study has shown that irradiation when performed during the neonatal period, whether in combination with NTx or not, does not suppress the homograft response; this investigation has also confirmed the view that NTx alone does not inhibit the capacity of birds to respond to heterologous (Srbc) antigenic challenge.

Although several workers (Horton-Smith and Long, 1963; Pierce et al., 1963; Leathem and Burns, 1968) have demonstrated circulating antibodies in Eimeria infections, these immunoglobulins generally lacked protective properties. The mechanism of immunity in coccidiosis might be compared to that associated with pathogenic organisms which invade the mucosa of the alimentary tract. Here a local production of muco-antibodies (analogous, perhaps, to the IgA antibodies of mammals, Tomasi and Bienenstock, 1968) occurs in response to antigenic stimulation; these antibodies are passed out into the lumen before the serum titers can rise to appreciable levels. This view, however, is contradicted by the results of Herlich (1961) who was unable to detect antibody activity in the faeces or in tissue extracts of fowls infected with E. tenella or E. acervulina. Horton-Smith and Long (1963) also failed to detect protective immunoglobulins either on the surface of the sporozoites or in the caecal lumen of chickens immune to E. tenella. In view of these data, the possibility of an involvement of reaginic type of antibodies (Augustin and Ridges, 1963) might be considered although information on the functional involvement of immunoglobulins in coccidial infections is very scanty.

It is tempting to speculate that the failure of 800 rads gamma irradiation to induce a marked increase in

oöcyst production may be related to an inhibitory effect of radiation on the relatively high rate of mitotic activity characteristic of the mucosal epithelium, thus reducing the availability of newly-formed cells for the invasive stages of the parasite; a high metabolic activity in the mucosa of the chick's intestine is indicated by the finding of Imondi and Bird (1966) that cells here are replaced approximately every 48 hours. Orlov (1966) has reported that radiation produces a marked depression in the biosynthesis of DNA in the small intestine. It is obvious that any interference in the metabolism of these cells after infection could inhibit the development of coccidia by depriving the parasite of a required intracellular environment characterized by a high level of synthetic activity.

The immunosuppressive treatments used in these experiments were not successful in altering the site specificity of Eimeria. A conflicting report has been presented by Long (1970) who was able to induce schizogonic development of E. tenella in the mid-intestinal region and the intrahepatic bile duct by means of treatment with the corticosteroid dexamethasone. However, since corticosteroids are capable of interfering with a great variety of physiological activities, his data do not necessarily imply that the change in location of the parasite was due to the

suppression of immune mechanisms. It might be added here that E. tenella infection has been established in other tissues without the use of immunosuppressive agents: E. tenella, E. brunetti and E. mivati are able to complete their endogenous cycle on the chorioallantoic membrane of the chick embryo (Long, 1965, 1966). E. tenella has also been shown to be capable of undergoing its asexual development in monolayer cultures of mammalian fibroblasts, mammalian epithelial cells and avian fibroblasts (Patton, 1965). On the other hand, since these tissues cannot be considered immunologically competent, they may be equivalent to cases of tissues where immunosuppression has been established.

VI. SUMMARY AND CONCLUSIONS

The present results confirm the view of several investigators that the bursa of Fabricius and the spleen play significant roles in the processes involved in circulating antibody production in the chicken. Clear evidence of the importance of the prenatal bursa is indicated by the abolition of circulating antibody synthesis to sheep red blood cells by hormonal bursectomy. The finding that neonatal removal of the bursa produces only a partial suppression of the antibody titer seems to indicate that the transfer of cells with immunological potential from the bursa to the secondary lymphoid sites takes place before hatching. This view is supported by the demonstration in this study that neonatal splenectomy results in a marked inhibition of the humoral antibody response. The more severe effect of immunoglobulin synthesis produced by splenectomy in the neonatal period than at three weeks of age might imply that there are other secondary lymphoid tissues which are capable of antibody production in the older bird. The enhancement of antibody titer produced by antibursal globulin treatment in experimental Series C has been difficult to explain,

and can only be clarified by further studies on the specific effects of antibursal antibodies.

In contrast to the effect of bursectomy, neonatal thymectomy (NTx) produced no effect on the agglutinin titer against sheep red blood cells. This seems to support the view that the thymus is not involved in circulating antibody production. However, since maturation of lymphoid tissue in this organ takes place long before hatching, neonatal thymectomy may not be effective in abolishing the thymic influence. However, impairment of the humoral immune reaction obtained when NTx was preceded by cortisone injection into the embryo on the 9th, 12th or 14th day of incubation, in contrast to cortisone treatment alone, suggests that the thymus may have at least an indirect role in the immune mechanisms associated with the humoral reaction. It was also demonstrated here that a partial suppression of the circulating antibody titer takes place in fowls subjected to antithymus globulin treatment. In view of these data, it can be postulated that (1) the thymic environment is, in fact, necessary for the immunological maturation of the bursa-derived cells before emigration to the secondary lymphoid sites or that (2) the thymus serves as a source of immunologic precursor cells for other sites, including the bursa of Fabricius.

The established view that the primary role of the thymus is associated with cellular immune responses is once again supported by the data presented in this investigation. The finding that a delay in graft rejection can be obtained by neonatal thymectomy in combination with splenectomy is in accordance with the concept that the thymus and the spleen are functionally interdependent. Such an interaction has also been indicated by the reduction in splenic weight observed in antithymus globulin-treated chickens. Nonetheless, further studies on the ultra-structure of the lymphoid cells contained within each of the lymphoid organs during the different phases of the immune response will need to be done before definite conclusions can be made from these results.

The role of the thymus in graft rejection is again indicated by the impairment of immunological response obtained in neonatally thymectomized-irradiated birds, as opposed to the less marked effect produced by heavy doses of radiation alone. It may be worth mentioning that, since total body irradiation results in a generalized lymphoid depletion, manifested by a reduction in weight of all the lymphoid organs examined here, this technique is not a valid criterion for understanding the specific roles of these organs. In addition, the physiological basis of the

damage produced by pre-immunization radiation treatment on the immune mechanism has not been elucidated.

Present data on the effects of the various immunosuppressive techniques on Eimeria tenella infection suggest that the mechanisms involved in host resistance may be local phenomena in the gastro-intestinal tract of chickens comparable to reactions produced by reagin-type antibodies or to the muco-antibody (secretory IgA?) response observed in mucosal infections caused by pathogenic micro-organisms. An apparent role of thymus-dependent cell-mediated immune reactions in coccidial infection was indicated by an enhanced oöcyst production, presumably resulting from the suppression of immunity in chickens subjected to neonatal thymectomy plus heavy doses of gamma irradiation; this treatment was also found to inhibit the rejection of skin homografts. Further support for the importance of the thymus is provided by the enhancement of the infective process induced by antithymus globulin treatment.

In view of the fact that antibursa globulin treatment also resulted in an increase in oöcyst production, the role of the bursa of Fabricius in immune reactions against Eimeria infection cannot be completely overlooked.

It is tempting to conclude from these results that the immune mechanisms associated with E. tenella infection

are local reactions under the direct control of "central lymphoid organs" and that the role of the spleen, if any, is a minor one. The nature of this regulation is still a matter of speculation. It may very well be that this control is a complex phenomenon involving non-specific factors of the inflammatory response, the physiological integrity of caecal cells under the influence of the parasitic invasion, genetic factors and, in part, the immunological potential of the host.

VII. AUTHOR'S CLAIM TO A CONTRIBUTION TO KNOWLEDGE

As far as is known to the writer, the following data are original contributions:

1. A marked suppression of circulating antibody synthesis against sheep erythrocytes by neonatal splenectomy and by the prenatal injection of cortisone acetate followed by thymectomy or bursectomy in the enonatal period.

2. A significant impairment of the hemagglutinin response and the homograft reaction by neonatal thymectomy or neonatal bursectomy in combination with heavy doses of gamma radiation on the day before antigen administration.

3. A marked inhibition of the humoral reaction against sheep red blood cell antigen and acceptance of homografts by treatment with heavy doses of gamma rays on the day before antigen administration.

4. Investigation on the influence of various doses of the purine analogue, azathioprine, on the sheep red blood cell hemagglutinin reaction in the fowl.

5. Studies on the effects of antithymus and antibursa globulin treatments on hemagglutinin production and skin homograft response in the domestic fowl.

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Appendix table 1.a. Statistical analysis of the $\log_2$ hemagglutinin titers of NTx, HBx (Tp 2.5), NBx, NSx and control groups of chickens, sacrificed at 5 weeks of age (Series A, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	47.6	4	11.9	1.69*
Within samples	218.4	31	7.05	
Total	266	35		

Appendix table 1.b. Statistical analysis of the body weights of HBx (19 nt) and untreated chickens at 4 weeks of age (Series A, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.05	1	0.05	5.0*
Within samples	0.08	7	0.01	
Total	0.13	8		

Appendix table 1.c. Statistical analysis of the body weights of hormonally bursectomized (19 nt) and control chickens at 5 weeks of age

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.01	1	0.01	2.5*
Within samples	0.03	8	0.004	
Total	0.04	9		

*Not significant at the 0.05 level of probability

Appendix table 1.d. Statistical analysis of the $\log_2$ hemagglutinin titers in CA_9 oil + NTx, CA_9 oil + NBx, CA_{14} oil + NTx, CA_{10} oil + NTx and untreated groups at 5 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	80.19	4	20.05	6.82**
Within samples	61.81	21	2.94	
Total	142.00	25		

Appendix table 1.e. Statistical analysis of the weights of the thymus gland in CA_{10} oil, CA_{11} oil and untreated chickens at 5 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.57	2	0.285	3.56*
Within samples	0.59	7	0.080	
Total	1.16	9		

Appendix table 1.f. Statistical analysis of the spleen weights of CA_{10} oil, CA_{11} oil, CA_9 oil + NTx, CA_{10} oil + NTx, CA_{11} oil + NTx, CA_{14} oil + NTx and untreated birds at 5 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.71	6	0.118	2.95**
Within samples	0.88	20	0.04	
Total	1.59	26		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 1.g. Statistical analysis of the bursal weights of CA_{10} oil, CA_{11} oil, CA_9 oil + NTx, CA_{10} oil + NTx, CA_{11} oil + NTx, CA_{14} oil + NTx and untreated groups at 5 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	6.606	6	1.101	4.17**
Within samples	5.008	19	0.264	
Total	11.614	25		

Appendix table 1.h. Statistical analysis of the $\log_2$ hemagglutinin titers of CA_{12} oil + NBx, CA_9 alc + NTx and untreated birds sacrificed at 4 weeks of age (Series A, Experiment II)

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	4.5	2	2.25	1.37*
Within samples	30.83	10	3.08	
Total	35.33	12		

Appendix table 1.i. Statistical analysis of the thymic weights of CA_9 oil, CA_9 alc, CA_{12} oil + NBx and untreated groups of chickens at 4 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.33	3	0.11	0.38*
Within samples	2.87	10	0.29	
Total	3.20	13		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 1.j. Statistical analysis of the spleen weights of CA9 oil + NTx, CA9 oil, CA9 alc + NTx, CA9 alc, CA12 oil + NBx and untreated chickens at 4 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.81	5	0.162	0.428*
Within samples	1.89	23	0.082	
Total	2.7	28		

Appendix table 1.k. Statistical analysis of the bursal weights of CA9 oil + NTx, CA9 oil, CA9 alc + NTx, CA9 alc and untreated chickens at 4 weeks of age (Series A, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	1.06	4	0.266	1.68*
Within samples	4.098	26	0.158	
Total	5.158	30		

Appendix table 1.l. Statistical analysis of the $\log_2$ hemagglutinin titers of NTx + 700R/2d, 600R/2d, 500R/3 wk, 600R/3 wk, 700R/3 wk and control birds (Series A, Experiment III)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	50.15	5	10	3.4**
Within samples	48.55	17	29	
Total	98.70	22		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 2.a. Statistical analysis of graft survival in NTx + Spx, Spx and control chickens (Series B, Experiment I.a)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	70.3	2	35.15	2.04*
Within samples	120.8	7	17.26	
Total	191.1	9		

Appendix table 2.b. Statistical analysis of the thymus weights of Spx and control birds (Series B, Experiment I.a)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	9.0	1	9.0	1.24*
Within samples	29.0	4	7.25	
Total	38.0	5		

Appendix table 2.c. Statistical analysis of the effect of Spx alone, and NTx + Spx on the bursal weight (Series B, Experiment I.a)

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	3.43	2	1.72	0.379*
Within samples	36.29	8	4.54	
Total	39.72	10		

*Not significant at the 0.05 level of probability

Appendix table 2.d. Statistical analysis of the homograft survival in chickens subjected to 500, 600, 700 or 800R gamma rays during the neonatal period (Series B, Experiment I.b)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	3.3	3	1.1	0.468*
Within samples	4.7	2	2.35	
Total	8.0	5		

Appendix table 2.e. Statistical analysis of the graft survival in NTx + 700R/2d, NTx + 600R/2d and control chickens (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	101.2	2	50.6	1.45*
Within samples	69.7	2	34.85	
Total	170.9	4		

Appendix table 2.f. Statistical analysis of the body weights of NTx + 700R/2d, NTx + 600R/2d and control chickens (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	949	2	475	7.09*
Within samples	133	2	67	
Total	1082	4		

*Not significant at the 0.05 level of probability

Appendix table 2.g. Statistical analysis of the graft survival in NTx + 700R/3 wk, 800R/3 wk and control birds (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	361	3	120.33	0.36*
Within samples	1339.7	4	335	
Total	1700.7	7		

Appendix table 2.h. Statistical analysis of the body weights[1] of 800R/3 wk and control chickens (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.06	1	.06	4.62*
Within samples	0.04	3	.013	
Total	0.10	4		

[1]Original data transformed into $\log_{10}$

Appendix table 2.i. Statistical analysis of thymus weights of CA_{12} + NBx, NBx, NBx + 600R/2d, NBx + 700R/2d, 800R/3wk and control birds at 10 weeks of age (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	18.53	5	3.71	0.839*
Within samples	22.11	5	4.42	
Total	40.64	10		

*Not significant at the 0.05 level of probability

Appendix table 2.j. Statistical analysis of spleen weights of CA_{12} + NBx, NBx, NBx + 600R/2d, NBx + 700R/2d, 800R/3wk and control chickens at 10 weeks of age (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	9.12	5	1.82	5.2**
Within samples	1.75	5	0.35	
Total	10.87	10		

Appendix table 2.k. Statistical analysis of bursal weights of 800R/3 wk and untreated chickens at 10 weeks of age (Series B, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	3.01	1	3.01	2.26*
Within samples	2.66	2	1.33	
Total	5.67	3		

Appendix table 2.l. Statistical analysis of the thymic weights of CBx, NBx + 700R/3 wk, CA_{12} oil, and control chickens at 11 weeks of age (Series B, Experiments II and III)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	24.36	3	8.12	2.63*
Within samples	12.37	4	3.09	
Total	36.73	7		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 2.m. Statistical analysis of the splenic weights of CBx, NBx + 700R/3 wk, 700R/2d, NTx + 700R/3 wk, CA_{12} oil, and control chickens at 11 weeks of age (Series B, Experiments II and III)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	43.72	5	8.74	2.78*
Within samples	18.82	6	3.14	
Total	62.54	11		

Appendix table 2.n. Statistical analysis of the bursal weights of NTx + 700R/2d, NTx + 700R/3 wk, CA_{12} + NTx, CA_{12} oil and control birds at 11 weeks of age (Series B, Experiments II and III)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	21.62	4	5.41	3.44*
Within samples	9.43	6	1.57	
Total	31.05	10		

*Not significant at the 0.05 level of probability

Appendix table 3.a. Statistical analysis of the $\log_2$ hemagglutinin titers of the various groups of ATGG- and ABGG-treated chickens and of the control group (Series C, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	180.82	8	22.6	2.86*
Within samples	63.18	8	7.9	
Total	244.00	16		

Appendix table 3.b. Statistical analysis of the thymic weights of the four ATGG groups and control birds of the same age (Series C, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	47.62	4	11.86	1.29*
Within samples	46.32	5	9.26	
Total	93.94	9		

Appendix table 3.c. Statistical analysis of the weights of the spleen in the four ATGG-treated groups and control of the same age (Series C, Experiment II)

Analysis of Variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.63	4	0.16	0.36*
Within samples	2.25	5	0.45	
Total	2.88	9		

*Not significant at the 0.05 level of probability

Appendix table 3.d. Statistical analysis of the bursal weights of the four ATGG-treated groups and control group of the same age (Series C, Experiment II)

Analysis of Variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	6.43	4	1.61	0.49*
Within samples	16.30	5	3.26	
Total	22.73	9		

Appendix table 3.e. Statistical analysis of thymic weights in the four ABGG-treated groups and the control group of fowls at 10 weeks of age (Series C, Experiment II)

Analysis of Variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	20.65	4	5.16	0.58*
Within samples	44.66	5	8.93	
Total	65.31	9		

Appendix table 3.f. Statistical analysis of the splenic weights of the four ABGG-treated groups and one control group of chickens at the age of 10 weeks (Series C, Experiment II)

Analysis of Variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.46	4	0.115	0.099*
Within samples	5.78	5	1.16	
Total	6.24	9		

*Not significant at the 0.05 level of probability

Appendix table 3.g. Statistical analysis of the bursal weights of the ABGG-treated and control groups of chickens at 10 weeks of age (Series C, Experiment II)

Analysis of Variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	5.4	4	1.35	0.053*
Within samples	12.77	5	2.55	
Total	18.17	9		

*Not significant at the 0.05 level of probability

Appendix table 4.a. Statistical analysis of the bursal weights of the NTx + 700R/3 wk and control groups of chickens at 13 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	11.12	1	11.12	28.51**
Within samples	1.95	5	0.39	
Total	13.07	6		

Appendix table 4.b. Statistical analysis of the spleen weights of the NTx + 700R/3 wk and control groups of chickens at 13 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	2.668	1	2.668	23.61**
Within samples	0.563	5	0.113	
Total	3.231	6		

Appendix table 4.c. Statistical analysis of the ($\log_{10}$) body weights of the NTx + 700R/3 wk and control groups of chickens at 13 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.04	1	0.04	6.67**
Within samples	0.03	5	0.006	
Total	0.07	6		

**Significant at the 0.05 level of probability

Appendix table 4.d. Statistical analysis of the ($\log_{10}$) oöcyst counts from the NTx + 700R/3 wk and control groups of chickens infected with 1000 oöcysts at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	9.80	1	9.8	2.48*
Within samples	90.93	23	3.95	
Total	100.73	24		

Appendix table 4.e. Statistical analysis of the bursal weights of the NTx + 700R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.41	1	0.41	0.157*
Within samples	5.23	2	2.61	
Total	5.64	3		

Appendix table 4.f. Statistical analysis of the spleen weights of the NTx + 700R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	1.146	1	1.146	2.795*
Within samples	0.820	2	0.410	
Total	1.966	3		

*Not significant at the 0.05 level of probability

Appendix table 4.g. Statistical analysis of the ($\log_{10}$) body weights of the NTx + 700R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.006	1	0.006	0.308*
Within samples	0.039	2	0.020	
Total	0.045	3		

Appendix table 4.h. Statistical analysis of the $\log_2$ hemagglutinin titers of sera from the NTx + 700R/3 wk and control groups of chickens injected at 3 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	25.10	1	25.10	14.77**
Within samples	13.63	8	1.70	
Total	38.73	9		

Appendix table 4.i. Statistical analysis of the ($\log_{10}$) oöcyst counts of the NTx + 700R/3 wk and control groups of chickens infected with 1000 oöcysts at 3 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	4.15	2	2.08	0.695*
Within samples	104.63	35	2.99	
Total	108.78	37		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 4.j. Statistical analysis of the thymus weights of the 800R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.01	1	0.01	0.0003*
Within samples	181.20	6	30.20	
Total	181.21	7		

Appendix table 4.k. Statistical analysis of the bursal weights of the 800R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	15.13	1	15.13	54.42**
Within samples	1.67	6	0.278	
Total	16.80	7		

Appendix table 4.l. Statistical analysis of the spleen weights of the 800R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	1.38	1	1.38	15.862**
Within samples	0.52	6	0.087	
Total	1.90	7		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 4.m. Statistical analysis of the (log_{10}) body weights of the 800R/3 wk and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.072	1	0.072	24**
Within samples	0.019	6	0.003	
Total	0.091	7		

Appendix table 4.n. Statistical analysis of the log_2 hemagglutinin titers of sera from the 800R/3wk and control groups of chickens (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	13.30	1	13.3	30.93**
Within samples	3.43	8	0.43	
Total	16.73	9		

Appendix table 4.o. Statistical analysis of the (log_{10}) oöcyst counts of chickens treated with 800R/3 wk and of control birds infected with 100 oöcysts (Series D, Experiment I)

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.56	1	0.56	0.467*
Within samples	14.42	12	1.20	
Total	14.98	13		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 4.p. Statistical analysis of the thymus weights of the HBx-treated and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	6.58	1	6.58	1.916*
Within samples	13.74	4	3.435	
Total	20.32	5		

Appendix table 4.q. Statistical analysis of the spleen weights of the HBx-treated and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.663	1	0.663	2.51*
Within samples	1.056	4	0.264	
Total	1.719	5		

Appendix table 4.r. Statistical analysis of the ($\log_{10}$) body weights of the HBx-treated and control groups of chickens at 10 weeks of age (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.022	1	0.022	4.4*
Within samples	0.020	4	0.005	
Total	0.042	5		

*Not significant at the 0.05 level of probability

Appendix table 4.s. Statistical analysis of the (log_{10}) oöcyst counts from chickens subjected to HBx and from normal birds infected with 1000 oöcysts (Series D, Experiment I)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.003	1	0.003	0.001*
Within samples	64.302	23	2.796	
Total	64.305	24		

*Not significant at the 0.05 level of probability

Appendix table 4.t. Oöcyst counts of 10-week-old chickens subjected to neonatal thymectomy plus gamma irradiation at 3 weeks and of control birds (dose: 1000 oöcysts per bird) (Series D, Experiment I)

Day after infection	No. of oöcysts produced per chicken per day	
	NTx + 700R/3 wk (4)	Control (4)
5	0	0
6	54,720	0
7	5,913,600	714,400
8	6,330,430	1,400,000
9	665,600	1,664,000
10	777,600	1,155,000
11	17,070	3,164,000
12	1,745,450	38,400
13	774,720	40,000
14	217,800	24,500
15	65,940	5,320
16	13,500	1,170
17	1,344	0
18	2,016	0
19	0	0
Total count	16,579,790	8,206,790

Appendix table 4.u. Oöcyst counts of 3-week-old chickens subjected to neonatal thymectomy and 700R gamma irradiation at 3 weeks of age and of untreated controls (dose: 1000 oöcysts per bird) (Series D, Experiment I)

Day after infection	No. of oöcysts produced per chicken per day	
	NTx + 700R/3 wk (6)	Control (2)
5	0	0
6	2,613	19,500
7	15,667,200	975,360
8	75,460,260	42,374,740
9	34,944,000	105,600
10	2,360,000	141,867
11	1,512,000	23,367,270
12	95,200	3,808,000
13	119,000	37,330
14	24,500	16,987
15	31,040	41,600
16	13,600	5,227
17	4,680	187
18	11,060	107
19	1,425	187
20	780	93
21	0	0
Total count	130,247,358	70,894,055

Appendix table 4.v. Oöcyst counts of chickens subjected to 800R gamma irradiation at 3 weeks of age and of control birds (dose: 100 oöcysts per bird)(Series D, Experiment I)

Day after infection	No. of oöcysts produced per chicken per day	
	800R/3 wk (4)	Control (6)
5	0	0
6	0	0
7	7,700	145,067
8	292,600	504,000
9	401,330	290,400
10	457,330	112,000
11	266,500	1,400
12	2,426	17,685
13	10,967	0
14	1,494	0
15	1,000	0
16	0	0
Total count	1,441,347	1,070,552

Appendix table 4.w. Oöcyst counts of chickens subjected to hormonal bursectomy and of untreated controls (dose: 1000 oöcysts per bird) (Series D. Experiment I)

Day after Infection	No. of oöcysts produced per chicken per day	
	HBx (5)	Control (3)
5	0	0
6	5,088	21,300
7	9,128,000	44,640,000
8	1,029,120	390,000
9	289,600	20,640
10	18,624	10,368,000
11	151,200	23,040,000
12	714,240	547,200
13	24,387	115,200
14	6,550	23,529
15	3,600	25,600
16	89,280	933
17	11,520	2,200
18	4,469	0
19	0	0
Total count	11,475,678	79,200,602

Appendix table 5.a. Statistical analysis of the weights of the thymus gland of ATG- and ABG-treated and control groups of chickens (Series D, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	4.34	2	2.17	2.49*
Within samples	8.67	10	0.87	
Total	13.01	12		

Appendix table 5.b. Statistical analysis of the bursal weights of ATG- and ABG-treated and control groups of chickens (Series D, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	1.87	2	0.935	0.87*
Within samples	10.78	10	1.078	
Total	12.65	12		

Appendix table 5.c. Statistical analysis of the spleen weights of ATG- and ABG-treated and control groups of chickens (Series D, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.06	2	0.03	0.108*
Within samples	2.79	10	0.279	
Total	2.85	12		

*Not significant at the 0.05 level of probability

Appendix table 5.d. Statistical analysis of the ($\log_{10}$) body weights of ATG- and ABG-treated and control groups of chickens (Series D, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	0.02	2	0.01	2*
Within samples	0.05	10	0.005	
Total	0.07	12		

Appendix table 5.e. Statistical analysis of the $\log_2$ HA titers of sera from ATG, ABG and control groups of chickens (Series D, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	17.15	2	8.58	6.5**
Within samples	15.79	12	1.32	
Total	32.94	14		

Appendix table 5.f. Statistical analysis of the ($\log_{10}$) oöcyst counts of ATG- and ABG-treated and control groups of chickens infected with 1000 oöcysts (Series D, Experiment II)

Analysis of variance

Source of variation	Sums of squares	Degrees of freedom	Variance estimate	"F" Value
Between samples	4.24	2	2.12	1.18*
Within samples	28.02	24	1.17	
Total	32.26	26		

*Not significant at the 0.05 level of probability
**Significant at the 0.05 level of probability

Appendix table 5.g. *E. tenella* oöcyst count of chickens treated with antithymus or antibursal serum and of control birds (dose: 1000 oöcysts) (Series D, Experiment II)

Day of infection	No. of oöcysts produced per chicken per day		
	ATG (6)	ABG (5)	Control (4)
5	0	0	0
7	1,358,400	2,770,000	80,770
8	372,000	725,000	112,000
9	525,000	1,125,000	163,800
10	88,000	623,080	136,980
11	113,070	636,000	135,500
12	41,140	238,000	186,510
13	6,690	30,200	121,935
14	1,506	5,016	124,520
15	432	23,150	12,960
16	116	0	1,440
17	0	0	0
Total count	2,506,354	6,175,446	1,076,415