THE EFFECT OF NEONATAL THYMECTOMY AND X-IRRADIATION ON THE LESIONS OF TUBERCULOSIS IN CHICKENS

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Dibakar Panigrahi
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ABSTRACT

THE EFFECT OF NEONATAL THYMECTOMY AND X-IRRADIATION ON THE LESIONS OF TUBERCULOSIS IN CHICKENS

By

Dibakar Panigrahi

The lesions of tuberculosis and the tuberculin reactions were studied in surgically thymectomized and thymectomized X-irradiated chickens infected at 10 weeks of age with mycobacteria.

In *Mycobacterium avium*-infected chickens the lesions at the sites of inoculation were somewhat smaller in the thymectomized X-irradiated chickens.

Tuberculin reactions were evident in almost all infected chickens 48 hours after injection of the tuberculin. Although individual chickens reacted with varying intensity, there was no detectable difference in tuberculin reactions among different groups. However, it is probable that the reactions were due to or complicated by an Arthus type sensitivity.

In thymectomized and thymectomized X-irradiated chickens there were varying degrees of lymphocytic depletion from the spleen.

All groups of chickens infected with a virulent strain of *M. avium* had granulomas in the liver, spleen, ileocecal junction, thymus and lungs. Although there was no essential difference in the cellular makeup of these lesions, caseation necrosis appeared to be less prominent in the thymectomized and thymectomized X-irradiated chickens. There were significantly

fewer caseous lesions in the hepatic sections of thymectomized and thymectomized X-irradiated chickens. There were more caseous lesions in the spleen than in the liver. Lesions at the sites of inoculation were seen in all birds infected with *M. avium*.

Among chickens infected with Group III mycobacteria, only small numbers had local lesions at the sites of inoculation, and there were no lesions of tuberculosis in the viscera. Thymectomy and/or X-irradiation did not detectably alter the susceptibility to Group III organisms.

In chickens infected with *Mycobacterium bovis* there were no lesions at the sites of inoculation or in the viscera.

With the present surgical technique, complete thymectomy in all birds was virtually impossible to accomplish. Sometimes the thymic tissue bordered the thyroid grossly and often extended posterior to this gland. Microscopically, thymic lymphocytes were seen adjacent to the thyroid follicles with no sharp line of demarcation between the two.

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By

Dibakar Panigrahi

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pathology

1970

To my father

Sri. Golakbehari Panigrahi

and to my late mother

Ashamani Panigrahi

To my father

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and to my late mother

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ACKNOWLEDGEMENTS

The author would like to express his gratitude to the following persons for their contribution to this study.

To Dr. G. L. Waxler, major professor, for his guidance and encouragement during the entire period of my study here.

To Dr. V. H. Mallmann for providing facilities for this work and for all of her valuable advice and suggestions.

To Dr. C. C. Morrill, Chairman of the Department of Pathology, Michigan State University, for encouraging me to pursue graduate studies in the department and for his valuable suggestions.

To Drs. R. F. Langham and V. L. Sanger for their useful advice and guidance.

To Mrs. I. S. Fauser, who has not only been my research partner but is a wonderful friend.

To Dr. U. V. Mostosky, Michigan State University Veterinary Clinic, for his help in X-irradiation of chickens.

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INTRODUCTION AND OBJECTIVES

The mammalian thymus is responsible directly or indirectly for maturation and function of antibody-dependent immunity and cellular immunity. Glick *et al.* demonstrated in 1956 that the ability to produce antibody was seriously impaired in chickens by removal of the bursa of Fabricius, a hindgut lymphocytic organ. Since then the chicken has been used as an experimental model for the study of the mechanism of the immune response. It is now well established that the central lymphocytic tissue in chickens is composed of the bursa of Fabricius and the thymus. The bursa of Fabricius is responsible for the maturation of humoral immunity. The thymus is responsible for the development of delayed hypersensitivity reactions in tuberculin-type sensitivity, homograft rejection, graft versus host reactions and experimental allergic encephalomyelitis.

Tuberculosis is known as one of the most destructive diseases of man and animals, but the cause of tissue necrosis in tuberculosis is undetermined. No toxic material is known to be present in the tubercle bacilli, nor is any toxin capable of causing tissue necrosis known to be elaborated by these organisms *in vitro*. Infection with tubercle bacilli regularly induces delayed hypersensitivity to the organism. That part of the tissue necrosis in tuberculosis is due to tuberculo-sensitivity is strongly supported by several workers, including Rich (1951). Removal of the thymus from chickens, followed by X-irradiation, has been reported to reduce or eliminate the capacity to develop delayed hypersensitivity. The chicken

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should, therefore, be an ideal model to study the role of delayed sensitivity in the pathogenesis of tuberculosis.

The studies reported include chickens infected with *M. avium*, *M. bovis* and Group III mycobacteria. These organisms vary in their pathogenicity for chickens. They were chosen because the first causes extensive lesions in chickens, the second causes local lesions, and the response of chickens to the third organism is variable.

The origin of Group III mycobacteria still remains an enigma. Some workers believe that some Group III organisms are less virulent strains of *M. avium*. Their argument is based on the fact that both Group III and *M. avium* have similar colonial characteristics and biochemical properties and closely related but not identical antigens. They are differentiated consistently only by their ability to cause lesions in chickens; whereas *M. avium* is capable of causing progressive tuberculosis, Group III organisms cause a few limited lesions or no lesions.

The objectives of this study were:

1. To study the distribution of lymphocytes in the spleen and ileocecal junction and to determine the quantitative effects of thymectomy alone or thymectomy followed by X-irradiation on the population of peripheral lymphocytes of chickens.
2. To determine if thymectomy and/or X-irradiation would alter the susceptibility to various mycobacteria.
3. To determine if the lesions of tuberculosis were altered by thymectomy and/or X-irradiation.
4. To study the lesions at the site of intradermal inoculation.

REVIEW OF LITERATURE

Historical background. The thymus has been the subject of extensive investigations by scientists for many years. Most of the studies dealt with gross and microscopic anatomy, pathology and the physiological role of the organ. Since lymphocytes first make their appearance in the thymus of developing embryos, this organ has long been recognized as a major site of lymphopoiesis. In the past few decades, there has been an increasing interest in the role of thymus in immunogenesis. Clinical observations in man have led scientists to explore the association of thymic abnormalities with immunologic deficiency diseases. The fact that patients with acquired agammaglobulinemia have a high incidence of thymoma, that the thymus undergoes involution in acute infections and that its size is maximum in early life when immunological maturation occurs, are a few of the observations which implied its role in immune function.

Anatomy of the thymus. In man and most other animals, the thymus is located in the upper part of the thoracic cavity. In guinea pigs and chickens it is located in the neck area (Metcalf, 1964). In chickens, the thymus is composed of 14 separate lobes, 7 on each side of the neck (Burnet, 1962). According to Lucas and Stettenheim (1965), the thymus in chickens is composed of at least 10 lobes in total. In chickens, the thymic lobes are distributed superficially to the jugular vein, with the lobes near the thoracic inlet being very close to the thyroid gland (Lucas and Stettenheim, 1965).

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Microscopically, the thymus is divided into cortex and medulla. The cortex has a dense population of deeply stained lymphoid cells. The medulla is composed of few, lightly stained lymphocytes.

The primary lymphoid organs of chickens include the thymus and the bursa of Fabricius. The latter is located above the cloaca and is composed of numerous lymphoid nodules in which lymphocytes are closely packed (Burnet, 1962).

Embryonic development of the thymus. The thymus has been reported to grow rapidly in embryonic life and to attain considerable size in the neonatal period. During early postnatal life its weight steadily increases. Its weight in children reaches a maximum around 12 years of age, after which it undergoes gradual involution (Good *et al.*, 1964). In embryonated chicks, the development of lymphoid tissue of the thymus and bursa occurs at different time intervals. Lymphoid tissues of the thymus appear about the 12th to the 14th day of incubation (Papermaster and Good, 1962; Peterson and Good, 1965). According to Warner (1967), lymphopoiesis in the avian thymus occurs around the 9th to the 10th day of incubation.

In the bursa of Fabricius the lymphocytes appeared around the 16th to the 18th day (Ackerman and Knouff, 1959) and in the spleen around the 14th to the 15th day (Aspinall *et al.*, 1963) of incubation, respectively.

Immunologic maturation of the thymic and bursal cells occurs at different times. Peterson and Good (1965) postulated that the thymic lymphocytes attained maturity by the 17th day of incubation and that no further change occurred after hatching. The bursa-derived cells were larger and less differentiated at the time of hatching, and developed fully after hatching.

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Further work by Cain and co-workers (1968) confirmed this observation. These workers studied the immunologic competence of the bursal, splenic and thymic cells by injecting them into the allantoic veins of 14-day-old chick embryos. Splenomegaly in the embryo was used as the criterion for estimating the intensity of the graft versus host reactions. Cells from the spleen and thymus, not from the bursa, produced significant enlargement of the spleen. Splenic cells were 5 times as competent as thymocytes in their ability to induce graft-versus-host reaction. According to Takahashi (1967), the bursa-derived cells were not able to produce antibody *in situ* because they were immunologically immature.

Sherman and Auerbach (1966) maintained that both the thymus- and bursa-derived cell populations are mature at the time of hatching. Warner (1964) reported that in chickens the lymphocytes from the thymic cortex were immunologically incompetent and that the competent cells were present only in the medulla throughout life. The thymus underwent involution around 4 to 5 months of age and the associated changes involved cortical atrophy of the thymus.

The thymus- and bursa-derived cells represent 2 distinct groups of cells based not only on their morphology but also on their physiochemical properties. Deoxyribonucleic acid (DNA)-synthesizing cells in the bursa of Fabricius numbered twice that of cells in the thymus, as evidenced by their higher *in vitro* uptake of tritiated thymidine (Warner, 1965).

Szenberg and Shortman (1966) fractionated lymphocytes from chicken blood by density gradient centrifugation. There was a difference in the distribution of cells in the gradient depending upon their origin from the thymus or bursa.

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Although it is not clearly established how the thymus influences the peripheral lymphocytes, it is shown indirectly that the thymic lymphocytes are seeded from the thymus to the peripheral system (Kindred, 1935; Sainte-Marie and Leblond, 1963, 1964). In mammals, direct experimental evidence in support of this hypothesis was provided by Miller (1962), Murray and Woods (1964) and Nossal and Gorrie (1964).

Miller (1962) showed, by transplantation of thymus grafts bearing chromosomal markers, that some of the cells in the spleen of thymectomized (Tx) recipients were of donor origin.

Murray and Woods (1964) and Nossal and Gorrie (1964), in separate experiments, injected tritiated thymidine into the thymus glands. By using this technique they were able to detect some labeled thymocytes in the peripheral lymphocytic tissues.

In chickens indirect experimental evidence in this regard has been provided by Woods and Linna (1965) and Warner (1965). Using a combination of local labeling of bursal cells with tritiated thymidine and bursectomy, Woods and Linna found that there was a transport of cells to the spleen and thymus from the bursa. Warner (1965) injected labeled bursal and thymic cells into normal chickens. The concentration of the thymic cells in the spleen was approximately 7 times larger than that of the bursal cells.

The thymus in mammals may also exert its influence through elaborating a substance, probably a diffusible humoral one. Thymus tissue contained in cell-impermeable diffusion chambers, when left inside the peritoneal cavities of thymectomized X-irradiated (Tx-X) mice, was able to reconstitute the immune mechanism. Such mice did not have any signs of immunologic

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deficiency (Osoba and Miller, 1963, 1964; Wong *et al.*, 1966). However, this unidentified substance was incapable of completely correcting the lymphopenia seen in thymectomized animals (Cooper *et al.*, 1967).

There is some evidence to suggest that, in chickens, the bursa of Fabricius may exert its influence on the peripheral system by a humoral substance. Glick (1960) reported that the effects of bursectomy could be partially corrected by injecting a saline extract of acetone-dried bursa. This was the first indication that a humoral substance in addition to bursal cells was probably necessary for the bursa of Fabricius to exert its influence on the peripheral lymphocytic tissue. Further evidence in support of this hypothesis has been provided by Isakovic and co-workers (1963), Jankovic and Leskowitz (1965) and St. Pierre and Ackerman (1965), who reported that the immunologic deficiency in bursectomized (Bx) chickens was corrected to a certain extent by bursa implantation from other chickens. Immunologic deficiency could also be corrected by implanting millipore chambers containing bursae from young chickens into the peritoneal cavity of Bx chickens.

Cooper *et al.* (1966b) reported that the germinal centers, plasma cell population and gammaglobulin synthesis in bursectomized X-irradiated (Bx-X) chickens could be corrected by injection of autologous bursal cells.

Takahashi (1967) was of the opinion that the bursa of Fabricius has a dual function in providing the initial supply of lymphoid cells to the peripheral tissue and in producing a humoral substance which is responsible for the proliferation and immunologic maturity of the migrating cells.

Relationship of the thymus to other lymphoid organs. The peripheral lymphocytic tissue in rodents is dependent on the thymus for its supply of lymphocytes, at least in the neonatal period. This was reported by Miller *et al.* (1962) from several studies. There was a marked lymphopenia in mice thymectomized at birth. The lymphocyte levels reached as low as 90% below the control during the third and the fourth months. The spleens of Tx mice were smaller in size. There was marked alteration in the histologic appearance of the organ as revealed by inactive follicles, decreased cell population and small numbers of mitoses. Some of the changes noted in the spleen were also present in the lymph nodes and in the Peyer's patches of the intestine (Miller *et al.*, 1962). Histologic alterations in the spleen and lymph nodes of mice thymectomized at birth have also been reported by Good and associates (1962).

Neonatally Tx rats also had lymphocytic depletion of the spleen and lymph nodes (Waksman *et al.*, 1962), and there was a significant reduction in the weight of the lymph nodes when examined at 68 days of age (Schooley and Kelly, 1964). Germinal centers and plasma cells, however, were unaffected by thymectomy (Waksman *et al.*, 1962).

Chickens do not have lymph nodes, and the peripheral localization of the lymphocytes occurs primarily in the spleen and in the wall of the gastrointestinal tract. In the spleen the cells derived from the thymus are located in the white pulp which surrounds the small arteries and arterioles and around the Schweigger-Seidel sheaths of the red pulp. The germinal centers of the spleen are, on the other hand, composed of large and medium-sized lymphocytes mixed with variable numbers of reticular cells and lymphoblasts (Jankovic and Isakovic, 1964). These cells are believed to originate in the bursa of Fabricius (Cooper *et al.*, 1965). The germinal centers (also called lymphatic nodules by Jankovic and Isakovic, 1964) are encircled by a thin layer of connective tissue.

In the digestive tract, the lymphocytic tissue is present in variable amounts in the esophagus, at the junction of the proventriculus and esophagus, the proventriculus, the area between the gizzard and duodenum, the duodenum, the cecum and the rectum (Calhoun, 1954). However, the largest accumulation of lymphocytes occurs at the ileocecal junction. At this site, the lymphocytes are arranged in nodules and in loose aggregates.

In the chicken, the thymus has been reported to play a significant role in the development and maturation of widespread cell populations that are characterized by small lymphocytes. The white blood cell counts revealed a marked decrease in the number of small lymphocytes in the peripheral blood of Tx (Warner and Szenberg, 1962; Isakovic and Jankovic, 1964; Longenecker and Breitenbach, 1969) and Tx-X chickens (Cooper *et al.*, 1966a). Histologic examination revealed marked depletion of small lymphocytes from the white pulp of the spleen of Tx (Jankovic and Isakovic, 1964) and Tx-X chickens (Cooper *et al.*, 1965; Cooper *et al.*, 1966a) and from the cecum (Cooper *et al.*, 1966a) of Tx-X chickens.

Neonatal thymectomy alone did not cause any striking depletion of peripheral small lymphocytes. To achieve a reduction of total lymphocytes in Tx chickens, X-irradiation was recommended by Cooper *et al.* (1966a). Lymphoid depletion from the bursal follicles of neonatally Tx chickens was reported by Jankovic and Isakovic (1964). Although their results were inconsistent, the cortex of the bursal follicles was depleted, and only a few discontinuous layers of undifferentiated cells were present. Cells with basophilic cytoplasm were present in the medulla of the follicles.

Removal of the bursa of Fabricius did not affect the lymphocyte levels of peripheral blood (Isakovic and Jankovic, 1964; Cooper *et al.*, 1966a). Thymectomy did not influence the plasma cell numbers (Jankovic and Isakovic, 1964).

Role of thymus in immune mechanism. Miller (1961) for the first time reported that mice thymectomized at 1 to 16 hours after birth were immunologically deficient. Skin grafts survived longer in these animals than in intact, sham-thymectomized mice and mice thymectomized at 5 days of age. Thymectomy of newborn mice was associated with a decrease in their capacity to produce serum antibodies. Removal of the thymus from adult mice had little or no significant effect on antibody production.

Further studies in rabbits (Archer and Pierce, 1961; Good *et al.*, 1962), in mice (Martinez *et al.*, 1962), in rats (Arnason *et al.*, 1962; Jankovic *et al.*, 1962) and in hamsters (Sherman *et al.*, 1964) have shown that animals thymectomized at an early age exhibit striking immunologic deficiencies. These are characterized by a decreased capacity to express cellular and humoral immunity.

In mammals, the thymus is essential for the initial development, maintenance and repopulation of peripheral lymphocytic tissue. It plays a definite role in the ontogeny and phylogeny of the immune response.

The chicken is unique because its central lymphocytic tissue is composed of the thymus and the bursa of Fabricius. Accordingly, any conclusion drawn from mammalian experiments involving the thymus may not be fully applicable to chickens. Whereas in mammals both the cellular and humoral responses are affected by thymectomy, in chickens the thymus is mainly responsible for the production of mediator cells of delayed hypersensitivity.

I. Immunologic role of bursa of Fabricius in chickens. The cells derived from the bursa of Fabricius are responsible for antibody production.

A significant contribution to the field of immunology was made by Glick and associates in 1956, when they accidentally discovered that, of 9 chickens bursectomized at 12 days of age and injected later with *Salmonella typhimurium* 0 antigen, 6 died and the remaining 3 produced no detectable antibody. This provided preliminary evidence that the bursa of Fabricius, a hindgut lymphoid organ, plays a significant role in antibody production.

Bursectomy can also be achieved hormonally. Meyer *et al.* (1959) reported complete inhibition of the development of the bursa with reduction in the size of the thymus and spleen in chickens hatched from eggs treated with 19 nortestosterone on the 5th day of incubation. These chickens were immunologically deficient and failed to produce antibody to bovine serum albumin (BSA). All the control chickens had detectable antibody. The antibody response was depressed more in chickens hatched from 19 nortestosterone-treated eggs than in chickens in which the bursa was surgically removed at 1 week of age (Mueller *et al.*, 1960).

Spleens from hormonally Bx chickens were half the normal weight and there was a relative absence of lymphocytic tissue and plasma cells. The small lymphocytes in the peripheral blood, however, were present in normal numbers in most of the hormone-treated chickens. Atrophy of the thymic cortex occurred in 40% of the treated birds (Szenberg and Warner, 1962). The authors postulated immunologic dissociation of the thymus and the bursa. They believed that cells of the bursa of Fabricius were responsible for the production of circulating antibody and delayed hypersensitivity and cells of the thymic cortex were responsible for the mediation of homograft reaction and graft versus host reaction.

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Spleens from hormonally Bx chickens were half the normal weight and there was a relative absence of lymphocytic tissue and plasma cells. The small lymphocytes in the peripheral blood, however, were present in normal numbers in most of the hormone-treated chickens. Atrophy of the thymic cortex occurred in 40% of the treated birds (Szenberg and Warner, 1962). The authors postulated immunologic dissociation of the thymus and the bursa. They believed that cells of the bursa of Fabricius were responsible for the production of circulating antibody and delayed hypersensitivity and cells of the thymic cortex were responsible for the mediation of homograft reaction and graft versus host reaction.

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There is further evidence in support of the role of the bursa in antibody production (Chang *et al.*, 1958; Papermaster *et al.*, 1962; Isakovic *et al.*, 1963; Graetzer *et al.*, 1963; Cooper *et al.*, 1966a). Surgical or hormonal bursectomy did not completely abolish gammaglobulin synthesis (Cooper *et al.*, 1967). Chickens without the bursa could produce some humoral antibody, although the response was less than normal and the antibody was primarily of the IgM (gamma M) type. In contrast, Takahashi (1967) reported that hormonally Bx chickens were incapable of antibody production even after primary and secondary antigenic stimulation.

In addition to surgical bursectomy, posthatching and hormonal bursectomy, bursectomy can be performed on the chicken embryo. Suppression of primary and secondary antibody response was evident when the bursa was removed during the 19th day of incubation. In contrast, only the primary response was markedly depressed in chickens whose bursae were removed immediately after hatching. In these chickens the secondary responses were unaffected (Van Alten *et al.*, 1968; Cooper *et al.*, 1969). Their serum levels of IgG and IgM were unaffected. When the bursa was removed on the 19th day of embryonic life, reduction in the level of IgG only was evident. The circulating levels of IgG and IgM were markedly reduced when bursectomy was performed on the 17th day of incubation (Cooper *et al.*, 1969).

Chickens bursectomized on the day of hatching and subsequently exposed to an LD_{50} dose of X-irradiation did not produce detectable antibody when inoculated with BSA or *Brucella abortus* cells. No antibody response was evident even after intensive antigenic stimulation. Although most of the Bx chickens did not produce antibody under similar conditions, a few chickens from this group had positive antibody response. The control X-irradiated chickens produced antibodies to BSA and *B. abortus* (Cooper *et al.*, 1966a). Jankovic and Isakovic (1966) reported that surgically Bx chickens were unable to produce circulating antibody after primary immunization. However, they responded to a second antigenic stimulation and antibody levels increased following subsequent antigenic challenge. This rise in antibody titer was associated with an increase in the number of "pyroninophilic" cells in the spleen. There was an increase in the numbers of plasma cells in the cecal tonsils and in the intestinal wall. Surgically Bx chickens, however, were deficient in IgG immunoglobulins.

Bursectomy only on the day of hatching did not affect the numbers of germinal centers in the spleen and in the intestine. Bursectomized and X-irradiated chickens did not have germinal centers in these organs (Cooper *et al.*, 1966a). Reduction in the numbers of germinal centers in the spleen of chickens occurred when they were bursectomized surgically on the 18th, 19th and 20th day of incubation (Cooper *et al.*, 1969). However, bursectomy on the 17th day of embryonic life completely eliminated germinal centers from the tissues of chickens.

The numbers of plasma cells in tissues are also influenced by the bursa of Fabricius. Reduction in the number of plasma cells in Bx chickens has been reported (Szenberg and Warner, 1962; Jankovic *et al.*, 1963; Isakovic and Jankovic, 1964). According to Cooper *et al.* (1966a), the plasma cells from the spleen and intestine were absent only in Bx-X chickens and not in Bx chickens. Numbers of plasma cells in the tissues were also reduced when bursectomy was performed on the 17th day of incubation. Bursectomy at hatching did not affect the plasma cell population (Cooper *et al.*, 1969).

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These studies indicate that the bursa of Fabricius is responsible for the production and maturation of cells which mediate antibody production. Bursa-derived cells migrate from this organ to the peripheral system sometime before hatching. The bursa exerts its influence early in life. Bursectomy 8 to 10 weeks after hatching had no demonstrable effect on the chicken's normal response to antigen (Chang *et al.*, 1957). In man the thymus appears to function in the same way as the combination of the bursa and thymus in the chicken.

II. Immunologic role of thymus in chickens. Since the bursa is necessary for the development of humoral immunity, the role of the avian thymus in the ontogeny of the immune response is of interest. Numerous reports suggest that the thymus in chickens is responsible for production of lymphocytes that mediate delayed hypersensitivity reactions.

Clinically, functional dissociation of the immune response has been documented in man for a long time. In the Bruton type of agammaglobulinemia, there is complete failure of plasma cell formation and gammaglobulin production. These patients, however, are able to express delayed hypersensitivity reaction and reject homografts, but the process of rejection is slower (Cooper *et al.*, 1965).

Using chickens as an experimental model, a functional dissociation of the immune response was postulated (Szenberg and Warner, 1962) and later confirmed by several workers (Aspinall *et al.*, 1963; Jankovic and Isvaneski, 1963; Cooper *et al.*, 1965; Cooper *et al.*, 1966a; Cain *et al.*, 1968).

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Neonatal thymectomy and X-irradiation partially depressed the antibody response to BSA, *Salmonella paratyphi* A and ϕ X 174 phage (Marvanova and Hajeck, 1969).

Szenberg and Warner (1962) reported that some hormonally Bx chickens also had atrophy of the thymic cortex. These chickens with thymic atrophy could be separated from chickens without any thymic change by their altered immune response characterized by a longer graft survival. Using surgically Tx chickens, Warner and Szenberg (1962) provided evidence that thymectomy severely altered the chicken's ability for rejection of foreign skin grafts. Neonatal thymectomy prolonged homograft survival. In these chickens, the graft rejection occurred at various time intervals, always beyond the rejection period for normal chickens. Only 90 to 95% complete thymectomy was achieved since all the thymic tissue could not be removed because of the anatomic location of the thymus. However, there was a rough correlation between the homograft rejection time and the number of small circulating lymphocytes. That the avian thymus played a significant role in homograft reaction was confirmed by Aspinall *et al.* (1963).

Furthermore, Jankovic and Isakovic (1964) reported that thymus grafts survived for a longer period in Tx chickens than in controls. When neonatal thymectomy was followed by total body X-irradiation, the homograft survival was prolonged only in 5 of 12 chickens. Thymectomy alone did not lengthen the time of graft survival (Cooper *et al.*, 1966a).

The incidence of experimental allergic encephalomyelitis was significantly reduced in Tx chickens (Jankovic and Isvaneski, 1963). Bursectomized, thymectomized and unoperated chickens were injected with homologous and heterologous spinal cord suspensions with complete Freund's adjuvant through various routes. Clinical signs of ataxia and paralysis were evident in Bx chickens sooner than in the control chickens. Only 1 of 7 Tx chickens had ataxia. No Tx chickens developed paralysis, most of the Bx and control chickens did. In Tx chickens, the central nervous lesions were of minimal intensity and the delayed wattle reaction to spinal cord lipids and tuberculin was less intense. Cooper and associates (1966a) sensitized chickens with diphtheria toxoid in complete Freund's adjuvant. Only 2 of 14 Tx-X chickens gave a positive wattle reaction at 24 hours, and none of the chickens from this group manifested severe adjuvant reaction at the site of inoculation. All the control chickens had severe adjuvant reaction and gave positive wattle reaction at 24 hours. The results indicated that the thymus-dependent cells were responsible for mediation or expression of delayed hypersensitivity reactions.

Blaw , Cooper and Good (1967) also attempted to induce experimental allergic encephalomyelitis in Tx-X, X-irradiated, Bx-X (agammaglobulinemic) and control chickens. Clinical signs of the disease were not seen to the same degree in Tx-X chickens; all other groups developed symptoms. Only 4 of 9 Tx-X chickens had neurological derangement. Perivasculitis in the brain was evident in only 5 of 9 Tx-X chickens. All the chickens in the remaining 3 groups had various degrees of perivascular inflammatory change.

Delayed hypersensitivity. Hypersensitivity has been defined by Boyd (1966) as "an altered or heightened way of reacting to an antigen or hapten which has harmful effects on the body." The delayed type of hypersensitivity is differentiated from the immediate type primarily by the following features. The intradermal reaction in individuals with delayed hypersensitivity develops more slowly, there are no demonstrable circulating antibodies, and delayed hypersensitivity can be passively transferred only by mononuclear cells from sensitized individuals and not by serum (Rosen, 1968; Benacerraf and Green, 1969).

Lymphocytic cells are believed to be the mediators of delayed hypersensitivity (Chase, 1945; Najarian and Feldman, 1961; Waksman *et al.*, 1961; Kay and Rieke, 1963; Benacerraf and Green, 1969). Blood lymphocytes are 2 to 5 times more competent than the cells from lymphocytic tissue (Brent and Medawar, 1967).

Passive transfer of delayed hypersensitivity. Bail (1910) demonstrated that hypersensitivity to tuberculin could be established in normal recipients by cells transferred from tuberculin sensitive donors. Landsteiner and Chase (1942) were able to transfer delayed hypersensitivity to picryl chloride by peritoneal exudate cells from sensitized to normal guinea pigs. Chase in 1945 confirmed the earlier work of Bail by passively transferring cutaneous hypersensitivity to tuberculin with cells obtained from peritoneal exudate, spleen and lymph node of sensitive guinea pigs. Transfer of delayed hypersensitivity to pollen antigens was accomplished through blood lymphocytes (Slavin and Garvin, 1964). Using thoracic duct cells, Coe and co-workers (1966) could not find any direct evidence relating to the role of small lymphocytes as mediators of delayed reaction. In their experiment there was a better correlation between the number of large lymphocytes and the successful transfer of delayed hypersensitivity.

Mechanism of delayed hypersensitivity. Numerous studies have been conducted to elucidate the mechanisms of the delayed reaction. Although much information has accumulated, its mechanism is not fully understood.

I. *In vivo* studies. Previous workers showed that delayed hypersensitivity could be passively transferred by lymphocytic cells. They postulated that these cells were responsible for mediation of delayed response in the recipients. Recent evidence suggests that not all the transferred cells take part in the delayed reaction. The reaction can be triggered by a small number of sensitized cells, and the large number of mononuclear cells that accumulate at the reaction site in response to the antigen are of host origin (Turk, 1962; Turk and Oort, 1963; McCluskey *et al.*, 1963). By passive sensitization with labeled cells, Turk (1962) reported that about 3% of the cells present in a tuberculin reaction were of donor origin. McCluskey and associates (1963) were of the opinion that the great majority of the mononuclear cells present at the reaction site were nonspecifically sensitized and had undergone recent proliferation. Host cells were required for the development of delayed reaction (Coe *et al.*, 1966). Recipient animals were not passively sensitized if they were irradiated prior to transfer of sensitized cells. Such animals were incapable of exhibiting delayed skin reaction. This was probably due to the destruction of the precursor cells. Recent evidence indicates that the mononuclear cells that nonspecifically accumulate at the site of delayed reaction do originate from the stem cells of the bone marrow and reach the reaction site through the blood (Lubaroff and Waksman, 1967).

The mechanism whereby a few sensitized lymphocytic cells impart and evoke delayed hypersensitivity has not been clearly understood. Transfer of tuberculin sensitivity in man by cell-free extracts prepared from leukocytes of sensitized persons has been reported (Lawrence, 1955). This factor, known as transfer factor, was subsequently characterized as a low-molecular-weight (less than 10,000) dialyzable substance resistant to the action of DNase, ribonuclease (RNase) and proteolytic enzymes. Bloom and Chase (1967), however, were unable to transfer delayed hypersensitivity in guinea pigs with disrupted leukocytes.

II. *In vitro* studies. Studies to determine the specificity and mechanism of delayed reaction have utilized lymphocytes from sensitized individuals.

Lymphocytes collected from blood or lymph nodes of sensitized individuals undergo morphologic alterations in the presence of specific antigen. Accordingly, lymphocytes from tuberculin-sensitive persons and guinea pigs, respectively, when cultured in the presence of specific antigen, transformed into blast cells (Pearmain *et al.*, 1963; Mills, 1966). This reaction was immunologically specific and could be correlated with the state of hypersensitivity as determined by skin testing. This morphologic transformation was associated with an increase in DNA synthesis (Paul *et al.*, 1968) and is believed to represent one of the first steps in delayed hypersensitivity.

In 1932, Rich and Lewis noted that the cell migration from tissue explants and buffy coats of tuberculous guinea pigs was inhibited in the presence of tuberculin. Cell migration from explants taken from normal animals was not affected. This phenomenon was later studied by George and Vaughan (1962) by a modified procedure. Peritoneal exudate cells of sensitized guinea pigs failed to migrate from the capillary tube in the presence of sensitizing antigen. This migration inhibition phenomenon was considered to be related to delayed hypersensitivity. Migration of guinea pig peritoneal exudate cells was inhibited in the presence of sensitizing antigen (David *et al.*, 1964a). This *in vitro* inhibition of cell migration was believed to be caused by an interaction between the sensitized cells

and antigen, not by serum antibodies. When sensitized peritoneal cells were mixed with normal cells in different proportions, it was found that only a small percentage of sensitized cells was required to initiate migration inhibition phenomenon (David *et al.*, 1964b). Specific inhibition was prevented by addition of Puromycin (a suppressor of protein synthesis) to the medium. Active protein synthesis was essential for inhibition of macrophage migration (David, 1965). Peritoneal exudate cells, collected 2 to 5 days after intraperitoneal injection of an irritant, are composed of a mixed population of lymphocytes and macrophages. When tuberculin was added to the medium containing macrophages, separated by fractionation from peritoneal exudate cells of sensitized guinea pigs, migration was not affected. Addition of as little as 2% lymphocytes from the exudate of sensitive guinea pigs and tuberculin caused migration inhibition of macrophages from either sensitive or nonsensitive guinea pigs (Bloom and Bennett, 1966).

The mechanism whereby a few sensitized lymphocytes initiate delayed reaction *in vitro* is not understood. In 1966, Bloom and Bennett reported that *in vitro* migration of monocytes was inhibited by a nondialyzable factor released by the sensitized lymphocytes in the presence of sensitizing antigen. This factor with a molecular weight of 67,000 was named migration inhibitory factor (MIF). This substance was released from sensitized lymphocytes after interaction with antigen when the lymphocytes were collected from animals exhibiting only the delayed type of hypersensitivity (Bennett and Bloom, 1968). This factor, when added *in vitro* to macrophages from either sensitive or nonsensitive guinea pigs, inhibited their migration.

Thor and Dray (1968) transferred specific sensitivity *in vitro* with RNA-extracts from lymph node cells of hypersensitive individuals. This extract was nondialyzable and was not inactivated by deoxyribonuclease (DNase) or trypsin. This study has not been confirmed. The bulk of the evidence suggests that the antigen reacts with a few sensitive lymphocytes which then release nonspecific factors.

The transfer factor of Lawrence (1955) and the MIF of Bloom and Bennett (1966) seem to represent 2 separate factors produced by or released from the sensitized lymphocytic cells when they come in contact with the antigen.

III. Role of antibody. Antibody mediation of delayed response has been suggested, but delayed hypersensitivity cannot be transferred regularly with serum. The specificity of delayed sensitivity due to a "cell-bound factor" was suggested by Zinsser and Tamiya (1926), and Karush and Eisen (1962) maintained that a small amount of high affinity antibody may be involved in the mechanism of delayed sensitivity.

According to Heise and co-workers (1968), the *in vitro* migration inhibition was caused by an antibody cytophilic for macrophages.

Macrophages undoubtedly play a significant role in delayed reactions. Whether it is an immunologically specific role or only the effect of nonspecific factors liberated from lymphocytes is somewhat controversial. An antibody cytophilic for macrophages was demonstrated in the sera of guinea pigs inoculated with sheep red blood cells in complete Freund's adjuvant. This antibody was not present in animals that were sensitized with incomplete adjuvant (Boyden, 1964). Such parallelism between the development of delayed hypersensitivity and cytophilic antibody production has also been demonstrated by others (Berken and Benacerraf, 1966). The activity of cytophilic antibody was found in 7S gamma_2 globulins (Berken and Benacerraf, 1966; Gowland, 1968), although some activity was also present in other 7S gamma globulins (Gowland, 1968). However, the majority of workers have not been able to show such direct relationship and the bulk of evidence is that sensitive lymphocyte, but not antibody, is responsible for mediation of delayed hypersensitivity. The mode of the immunologically specific reaction between lymphocyte and antigen is not yet known.

Role of delayed hypersensitivity in the pathogenesis of tuberculosis. The role of delayed hypersensitivity in tuberculosis remains controversial as to whether it is the basis of acquired resistance or cellular immunity to tuberculosis, plays no significant role in resistance, or is harmful to the host. According to Lurie (1964), Uhr (1966), and Dannenberg (1968), delayed hypersensitivity may be either protective or harmful depending upon the dose of the antigen. At low concentration, the tuberculin-like products of the organism may stimulate macrophages so they can more ably inhibit or destroy the organisms. At higher concentration, necrosis of the macrophages and the surrounding tissue occurs (Dannenberg, 1968).

I. Beneficial effects. In 1891, Koch reported that tuberculous and normal guinea pigs reacted differently to the intradermal injection of tubercle bacilli. The response of the tuberculous animal was quicker and more severe at the site of injection than in the normal animal. There was necrosis and ulceration in the skin and the spread of the bacilli was inhibited. This immunologically specific hyper-reactivity, known as the "Koch Phenomenon", was considered by many as a beneficial mechanism, and they argued that the local tissue destruction was an attempt by the host to rid itself of infection. Römer (1908), a supporter of this hypothesis, pointed out that the effect of hypersensitivity could be harmful or beneficial depending on the number of organisms used for reinfection. When the number was large there was more tissue destruction. Smaller numbers of organisms caused less tissue damage. In the latter case, severe damage did not occur because of the protective effects of acquired resistance which

was unopposed by the low hypersensitive state of the animals. Krause (1926) was of the opinion that acquired resistance to tuberculosis was due to accelerated inflammation.

Because delayed hypersensitivity and acquired resistance to tuberculosis generally develop concomitantly, many believed that they were interrelated (Arnason and Waksman, 1964). This relationship has been very well illustrated experimentally by infecting animals that were immunized with tubercle bacilli of low virulence (Schwabacher and Wilson, 1938; Wells and Brooke, 1940; Dubos *et al.*, 1953a), with killed tubercle bacilli (Dubos *et al.*, 1953b; Weiss and Dubos, 1955) or with methanol extract from the bacilli (Weiss and Dubos, 1955). In all of these instances, a greater acquired resistance to tubercle bacilli was demonstrated in vaccinated animals than in controls. The interrelationship between hypersensitivity and immunity was further substantiated in a report by Wallgren (1953) involving BCG vaccinated children. Vaccinated children who failed to develop hypersensitivity also lacked acquired resistance to tuberculosis. Lurie and Dannenberg (1965) reported that under certain conditions strains of rabbits more resistant to tuberculosis also had higher degrees of delayed hypersensitivity than less resistant strains.

Arnason and Waksman (1964) stated,

> "No final conclusion can be reached as to the relation between tuberculin sensitivity and tuberculous immunity. The bulk of the evidence favors the view that it is a significant positive factor in immunity."

Dannenberg (1968), in a review article, stressed the importance of hypersensitivity in restricting local infection by airborne tubercle bacilli and reducing or preventing hematogenous spread. In small numbers, the bacilli are less injurious to macrophages, and probably stimulate cellular immunity.

The parallelism between delayed hypersensitivity and acquired resistance has not always been apparent. Rothschild *et al.* (1934) reported that desensitization of immunized guinea pigs by repeated injections of tuberculin did not impair their resistance to tuberculosis. This has been confirmed by other workers (Higginbotham, 1937; Birkhaug, 1939). The validity of this type of study has been challenged because skin hypersensitivity reappeared and desensitization probably had no effect on the allergic state of the animal (Wilson *et al.*, 1940; Mackaness, 1964). Mackaness (1964) emphasized that elimination of skin sensitivity did not necessarily lower the activity of macrophages which were responsible for acquired resistance.

Willis *et al.* (1938) could not find any experimental evidence in support of the previous report of Rothschild and co-workers (1934). The former workers found that animals desensitized with tuberculin also lost their resistance and died earlier from tuberculous pneumonia.

Lack of parallelism between hypersensitivity and acquired resistance was also evident from other studies in which skin sensitivity was demonstrable without any resistance to infection (Raffel, 1948), and resistance to infection was induced without cutaneous sensitivity to tuberculin (Weiss and Dubos, 1955).

Rich (1951) did not agree with the hypothesis that delayed hypersensitivity was protective to the host. Consequently, he wrote,

> "There had never been placed on record one single experiment or clinical observation that demonstrated that hypersensitivity is necessary for protection in any stage of tuberculosis or any other infection, under any condition whatsoever."

Boyd (1966) stated,

> "If hypersensitivity of the delayed type has any beneficial effect, it must be admitted that we do not know what it is...Consequently we must agree

> with Rich in thinking that the existence of the phenomenon of delayed hypersensitivity does not of itself show that the process is beneficial."

Nonetheless, delayed sensitivity can be induced without cellular immunity, but cellular immunity cannot be induced without delayed sensitivity.

II. Harmful effects. Tuberculosis has been one of the most destructive diseases of mankind since ancient times. To date, no potent toxin capable of damaging tissues in normal individuals has been identified from tubercle bacilli, and no evidence has been provided to support the fact that these organisms produce any such substance or substances *in vitro* (Rich, 1951).

In the early tuberculous lesions large numbers of tubercle bacilli have been seen in histiocytes and giant cells without any appreciable damage to these cells. However, concomitantly with the development of hypersensitivity, necrosis has been reported to take place (Lurie, 1934, 1964; Berthrong, 1970).

The fact that tubercle bacilli cause severe tissue damage in spite of no demonstrable toxic material has led to several theories to explain the mechanism of the tissue necrosis observed.

A. Caseation necrosis. Virchow (1858) believed that necrosis in tuberculosis was due to ischemia which resulted from the accumulation of dense cellular exudate, thereby blocking the blood supply. Arnason and Waksman (1964) believed that at least part of the necrosis was associated with impaired circulation, but this was not acceptable to Lurie (1964), who frequently found blood capillaries in the vicinity of caseous lesions in the lungs of rabbits.

Other workers provided experimental evidence in support of the theory that an increased oxygen supply was responsible for the caseation necrosis

seen in tuberculosis. The increased oxygen favored bacterial growth, thereby causing an increase in their number (Olson *et al.*, 1952). Lurie (1964) did not believe that increased oxygen tension in the tissues was essential for the growth of mycobacteria.

An immunological basis for tissue necrosis in tuberculosis has gained much support since the original discovery by Koch (1891) that tuberculous and normal animals behave differently to intradermal injections of tubercle bacilli or their products. The local tissue necrosis noted by Koch was interpreted by some to be harmful to the host.

Krause (1927) and Rich and McCordock (1929) considered caseation to be the result of the action of the tuberculin-like substance on the sensitized tissue of "allergic" individuals. The first experimental evidence in support of this hypothesis was provided by Rich and Lewis (1927-28), who showed that addition of tuberculin to cell cultures destroyed only those cells that were obtained from sensitized animals and not from normal animals. This work has been confirmed by several other investigators.

It is now believed by many that the degree of delayed hypersensitivity and the number of organisms present are important factors responsible for tissue destruction in tuberculosis (Rich, 1951; Canetti, 1955; Lurie, 1964).

The following arguments have been presented for the role of hypersensitivity in caseation.

First, caseation occurred earlier in the lungs of reinfected rabbits than in animals infected for the first time. The early necrosis in the former group was considered to be due to hypersensitivity (Canetti, 1955; Lurie, 1964).

Second, infected animals desensitized with tuberculin had less tissue necrosis than control animals when subjected to new infection (Rothschild

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Second, infected animals desensitized with tuberculin had less tissue necrosis than control animals when subjected to new infection (Rothschild

et al., 1934; Follis, 1938; Wilson *et al.*, 1940). *In vitro* cytotoxic effect of tuberculin was considerably less on splenic explants from desensitized animals than on those from hypersensitive ones (Kirchheimer and Weiser , 1948). However, killed bacilli caused more caseation in sensitive than in nonsensitive animals, but there was no correlation between the degrees of sensitivity and caseation (Olcott, 1939).

Third, enhancement of the hypersensitive state by using dead tubercle bacilli suspended in petrolatum as the sensitizing agent simultaneously increased the tendency for caseation (Sanez and Canetti, 1938).

Fourth, animals such as dogs, cats, horses, rats and mice have been known to develop low degrees of hypersensitivity. These animals also have less caseation in tuberculous lesions than other animal species with higher degrees of sensitivity (Canetti, 1955; Francis, 1958).

Canetti (1955) stated,

> "The facts indicate clearly, then, that caseation is in its essence a phenomenon of hypersensitivity, and that it is the presence of a large number of bacilli that sets it in motion."

Smithburn (1937) was of the opinion that the virulence of the organism played the most important role in caseation and that hypersensitivity was of minor importance. By using bovine tubercle bacilli of varying degrees of virulence, he found that there was greater tissue damage by virulent bacilli than by less virulent ones. Rich (1951) pointed out that virulence was not directly connected with the increased amount of tissue necrosis. It, however, could contribute to increased necrosis in 2 ways: first, by causing the organisms to multiply rapidly, thereby increasing their number and, secondly, by rapid induction of hypersensitivity.

B. Liquefaction. While caseous granulomas may undergo encapsulation or resolution, often liquefaction of the caseous mass takes place in

man and animals. This liquefaction plays an important role in the endogenous and exogenous spread of tuberculosis. Liquefaction may be due to any one of 4 factors--secondary infection of the lesion, polymorphonuclear cell invasion, presence of large numbers of tubercle bacilli, and proteolytic enzymes released from the damaged cells (Canetti, 1955). However, 2 theories have gained much support from the investigators that have worked on this problem.

1. Enzyme theory. It was believed by many, including Rich (1951), that liquefaction of the caseous material was due to the action of proteolytic enzymes. The source of these enzymes was again another controversial matter. Four possible explanations have been given in the literature.

a. Since acid-fast bacilli were almost always present in large numbers in the liquefactive lesions, some believed that the organisms were the source of the proteolytic enzymes. This was not acceptable to Wells and Long (1932), Rich (1951), and Weiss *et al.* (1954), since the proteolytic enzymes of tubercle bacilli were very weak and the number of bacilli in these lesions was not constant.

b. The polymorphonuclear cells invading the necrotic areas were probably the main source of proteolytic enzymes (Huebschmann, 1928; Rich, 1951). However, the proteinases from polymorphonuclear cells digested only 20% of the caseous tissue no matter whether the cells were obtained from normal or tuberculous rabbits (Tabachnick and Weiss, 1956).

c. Weiss *et al.* (1954), Tabachnick and Weiss (1956), and Long (1958) were of the opinion that to some extent liquefaction may be due to the action of enzymes diffusing from the surrounding inflammatory area into the caseous mass.

d. By histochemical methods, Dannenberg and co-workers (1968) showed that nucleases such as RNase and DNase were active in the area of caseation and liquefaction along with other lysosomal enzymes. The authors believed that the liquefactive process was carried out by these enzymes liberated from macrophages and polymorphonuclear cells.

2. Immunologic theory. It was the contention of Pagel (1936) that liquefaction in tuberculosis was related to the hypersensitive state of the individual. This was further substantiated by Ogawa *et al.* (1957) and Yamamura (1958), who reported that cavity formation occurred less frequently in desensitized than in sensitized animals.

Since in the same individual some lesions underwent liquefaction while others remained unchanged, Rich (1951) could not agree with the contention that hypersensitivity was in any way responsible for the liquefactive process. However, the author did not rule out the possibility of a local hypersensitivity reaction resulting from increased proliferation of tubercle bacilli. Long (1958) and Dannenberg *et al.* (1968) were of the opinion that hypersensitivity was at least partially responsible for liquefaction of the caseous tubercles.

C. Tubercle formation. Dienes, in 1936, reported that tubercles always developed in hypersensitive individuals as a response of the allergic tissue to the invading mycobacteria. This received some support from Olcott (1939) who, by injecting killed tubercle bacilli, showed that there was an increase in the number of tubercles in the sensitized animals. However, there was no correlation between the degree of hypersensitivity and the number of tubercles formed.

Several other works have emphasized that hypersensitivity was not essential for tubercle formation (Rich and McCordock, 1929; Vorwald, 1932;

Gardner, 1937; Rich, 1951; Lurie, 1964). Evidence in support of this hypothesis has come from experiments in which tubercles were demonstrated in animals as early as 3 days (Rich and McCordock, 1929) and 24 hours (Vorwald, 1932) after infection; silica injected intravenously caused tubercle-like lesions but did not induce hypersensitivity (Gardner, 1937). Finally, lipids and fatty acids from tubercle bacilli caused epithelioid cell granulomas without any hypersensitivity (Lurie, 1964).

Group III mycobacteria. The Group III mycobacteria differ from the classic pathogenic mycobacteria such as *M. tuberculosis* and *M. bovis* in that they do not produce progressive disease experimentally in guinea pigs or rabbits, although some Group III organisms may cause lesions in the bone and tendon sheaths of rabbits (Runyon, 1965). Their colonies are non-pigmented whether or not they are incubated with exposure to light. The optimum temperature for their growth is 35 to 37 C.; some may grow at 22 C., 30 C. and 44 C. (Mallmann *et al.*, 1964).

MATERIALS AND METHODS

Chickens. Eggs from Single Comb White Leghorn chickens were incubated at a dry-bulb temperature of 99 to 100 F. and a wet-bulb temperature of 85 to 86 F. in an International incubator with automatic rotation and controlled humidity. Two hundred four chickens were used in 4 experiments.

Thymectomy. Surgical thymectomies were performed under general anesthesia within 24 hours after hatching. A combined preparation of sodium thiopental (75%) and sodium pentobarbital (25%)* was injected intraperitoneally. The dosage varied as needed from 0.05 to 0.08 ml. The thymic lobes were exposed by a small incision on the dorsal aspect of the neck. Each lobe was removed carefully by small forceps after removing the adipose and connective tissue which surrounded the thymic lobes. Sham thymectomies were done in a similar manner except the thymic lobes were not displaced. When the operation was over, the incisions were closed with clamps. The surgery was conducted under aseptic conditions, and postsurgical treatment with antibiotics was not undertaken.

X-irradiation. Three-day-old chickens were irradiated with a General Electric Maxitron 300 X-ray machine. The conditions of irradiation were: 220 PKV, 20 m amp with 0.25 mmCu.+1.0 mm Al. added (with half value layer of approximately 0.25 mmCu) at a dose rate of 6.4 R/min. A total dose of 800 R was given in 125 min. in air.

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*Combuthal, Diamond Laboratories, Inc., Des Moines, Iowa.

Several experiments were conducted with altered dose levels to determine the $LD_{50/35}$. The doses used were 650 R, 750 R and 850 R. From the results, the $LD_{50/35}$ was estimated to be 800 R.

For X-irradiation, the chickens were placed in individual compartments in 4 cardboard boxes made in such a way that 100 chickens, 25 per box, were irradiated at the same time. The 4 boxes were arranged in a square and rotated at regular intervals in order to insure even exposure to X-ray. Using this procedure, the variation in the dose each chicken received was calculated to be less than 2% (Mostosky, 1966).

All the chickens were maintained under standardized conditions. Balanced ration and water were provided *ad libitum*.

Infection. Chickens from 4 different lots (Tx, Tx-X, control and X-irradiated) were included in each experiment. They were inoculated intradermally at an age of 10 weeks with viable cultures of mycobacteria. The dosage used in each case was 0.1 ml. (5 mgm. wet weight).

The cultures were as follows:

Mycobacterium avium - chicken origin

Mycobacterium bovis - swine origin (81-0)

Group III mycobacterium - bovine origin (50 B-0)

The Group III mycobacterium was classified according to Runyon (1959).

Leukocyte counts. Blood samples were collected in the morning from the wing vein and differential counts made (Fauser, 1969).

Tuberculin test. All the chickens were inoculated intradermally with 0.05 ml. of avian tuberculin (NADL St. Eliz. #7) in the wattle a few days before the end of each experiment. The results were recorded according to the degree of swelling 48 hours after inoculation. In one experiment readings were made at 2, 5, 27 and 48 hours.

Postmortem examination. At about 5 months of age, all chickens were killed by chloroform inhalation. The neck region was examined carefully for thymic remnants in the thymectomized chickens and for other possible changes in the thymus of nonthymectomized chickens. When thymic residua were detectable, the chicken was recorded as incomplete for thymectomy. Thyroid glands and tissues resembling thymus were fixed in 10% formalin for microscopic evaluation.

The inoculation sites in the skin were examined carefully for localized lesions and the approximate size of each lesion was measured.

All the viscera were examined for gross lesions. The approximate diameter of any lesions was measured. Gross alterations in the size of any organs were recorded. Portions of liver and spleen were collected for bacterial isolation. Sections of liver, spleen, lung, ileocecal junction, and thymus were fixed in 10% formalin for histopathologic examination. In order to avoid possible public health hazards associated with handling infected material, gross pictures were obtained after formalin fixation.

Histopathologic examination. Tissues were processed according to accepted methods and embedded in paraffin (Armed Forces Institute of Pathology, 1968). Sections were cut at a thickness of 5 microns and stained with New Fuchsin-Hematoxylin-Eosin stain (Willigan *et al.*, 1961). Tissues were examined for the following:

1. Normal lymphocytic cell population of the spleen and ileocecal junction and the effect of thymectomy on such tissues.
2. Completeness of thymectomy.
3. Lesions at the skin inoculation site.
4. Microscopic lesions in visceral organs.

The number of lesions from each section of liver and spleen was counted under low magnification. The presence or absence of acid-fast bacilli and various cell types in the granulomas were recorded. The number of granulomas with central necrosis was counted from each section, and an attempt was made to determine any differences in the type of lesion that could have resulted from the effect of thymectomy or X-irradiation. Sections were also examined for amyloid deposition. Selected sections were stained with Congo red (Armed Forces Institute of Pathology, 1968) and examined for amyloid under polarized light.

Bacteriologic technique. Tissues from the liver and spleen were ground in a mortar with a pestle in nutrient broth. An equal amount of 1 N NaOH was added to the tissue homogenate, and after 15 minutes at room temperature this was neutralized with 1 N HCl. The sample was centrifuged and the sediment seeded on 5 tubes each of Dubos oleic agar and Lowenstein-Jensen media. The tubes were incubated at 37 C. and examined for visible colonies on the first day of every month.

Statistical analyses. Some data were analyzed by the basic analysis of variance technique for cross classified data. Because the number of variables under study either exceeded 2 or analysis was further complicated by unequal sample sizes (Ruble *et al.*, 1968), the Michigan State University computer model CDC 3600 was used.* Transformation of data was done whenever indicated. A nonparametric sign test (Siegel, 1956) was used to determine the difference in the type of splenic lesions within each group of infected chickens.

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*Use of the Michigan State University computing facilities was made possible through support, in part, from the National Science Foundation.

RESULTS

The percent mortality due to various doses of irradiation for 3-day-old Tx and control chickens is given in Figure 1. The highest death rate of 79% was recorded when chickens were exposed to 850 R. Exposure to 750 R and 650 R caused a mortality of less than 50%.

The percent mortality and the mortality range in chickens exposed to an $LD_{50/35}$ dose of 800 R in 3 different experiments is presented in Figure 2.

The distribution of the treatments of the 174 chickens used in 3 different experiments is shown in Table 1. Unless otherwise indicated, the term "control" will be used to designate nonthymectomized, non X-irradiated chickens.

M. avium Experiment

Seventy-two chickens were used in this experiment. Of this number, 48 were infected with a virulent strain of *M. avium*. As seen in Table 2, 7 infected chickens and 1 uninfected chicken died at various time intervals before termination of the experiment at approximately 10 weeks after infection. Five of the 7 infected chickens that died belonged to the Tx-X group. They represented approximately 30% of the chickens used in this group. Three of the 5 dead Tx-X chickens had gross lesions of various sizes in the liver and spleen. These lesions were confirmed as tuberculous granulomas by histopathologic examination. The other 2 chickens died on

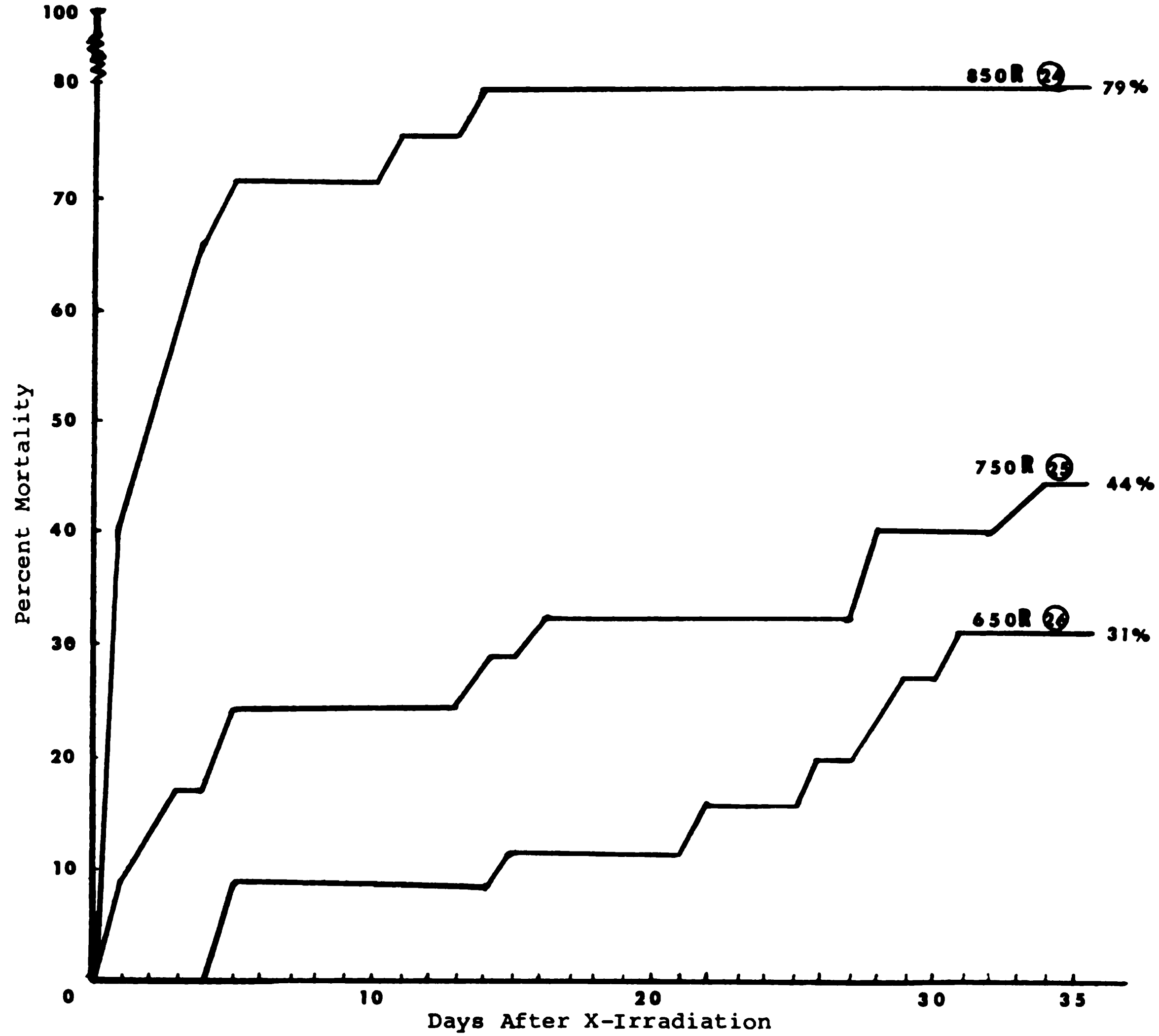

Figure 1. Percent mortality of 3-day-old thymectomized and normal chickens following X-irradiation. The circled numbers indicate the number of chickens represented by each line at the dose indicated.

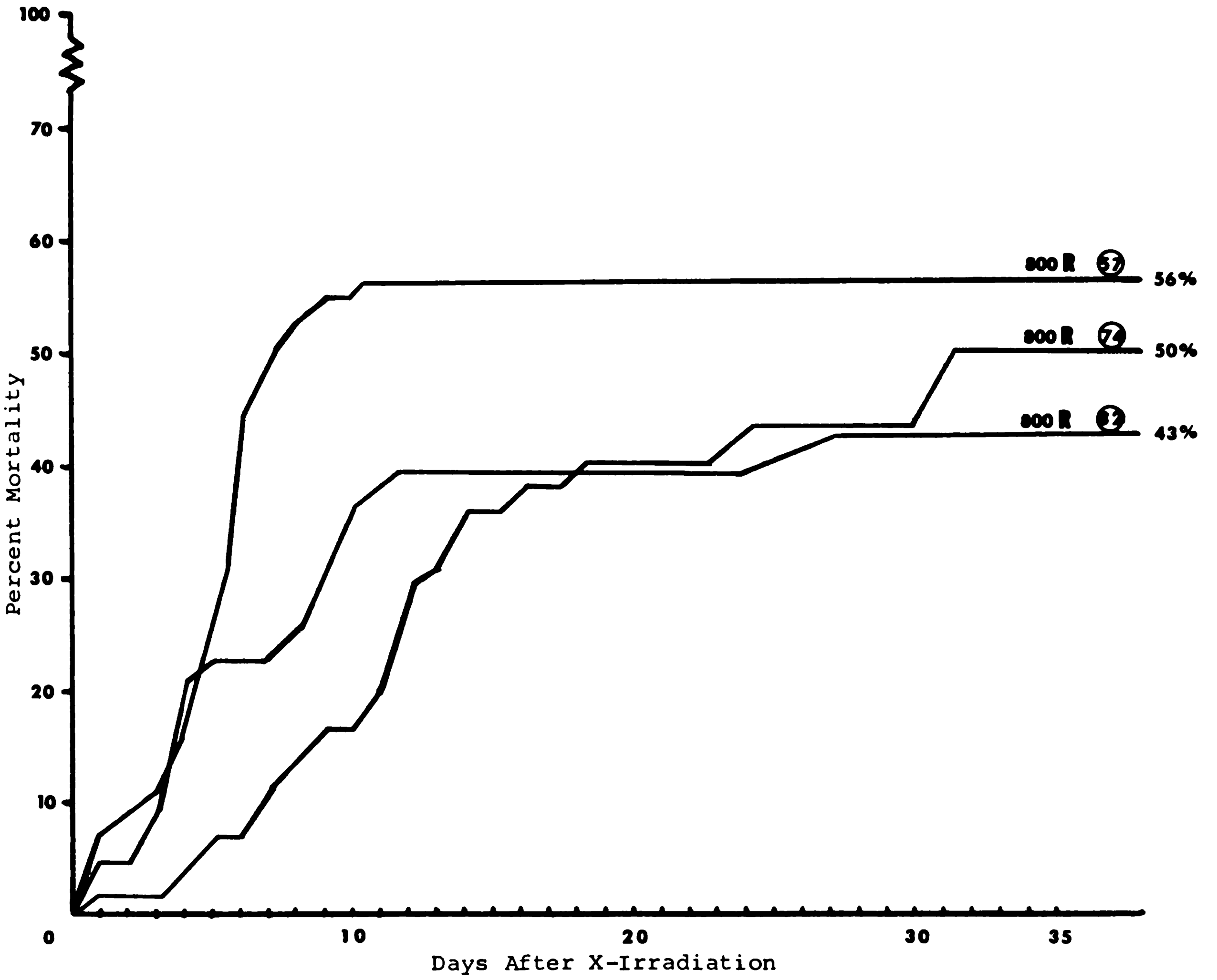

Figure 2. Percent mortality of 3-day-old thymectomized and normal chickens following 800 R X-irradiation. The circled numbers indicate the number of chickens represented by each line.

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Table 1. Distribution of various treatments among the chickens used in 3 different experiments

Treatment	*M. avium* experiment		Group III experiment		*M. bovis* experiment	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
Thymectomy	5	3	8	4	6	4
Thymectomy and X-irradiation	17	6	7	4	6	4
None	10	7	10	5	10	5
X-irradiation	16	8	10	5	9	5
Total	48	24	35	18	31	18

Table 2. Number of chickens alive and dead at the end of the *M. avium* experiment

	Tx	Tx-X	C	X
Infected	5*/0	12/5	9/1	15/1
Uninfected	3/0	6/0	6/1	8/0

*Numerator, no. of chickens alive; denominator, no. of chickens dead.

Abbreviations: Tx = thymectomized; Tx-X = thymectomized X-irradiated; C = control (nonthymectomized, non X-irradiated); X = X-irradiated.

the 18th day and 1-1/2 months after infection, respectively, and they did not have any gross or microscopic lesions of tuberculosis. The cause of their deaths could not be ascertained.

Two other infected chickens, one each from the X-irradiated and control groups, died before the end of the experiment. They had tubercles in the liver and spleen.

One control, noninfected chicken died. The cause of its death could not be determined by gross, microscopic or bacteriologic examinations.

From these results, the death rate in the Tx-X group appeared to be higher than in other groups. This was subjected to statistical analysis. Table 3 presents the analysis of the effects of thymectomy, X-irradiation and infection on the percent of chickens living at the end of the experiment. Since the data were expressed in percentages, arc sine transformation was indicated.

The 3-way analysis of the percent living at the end of the experiment indicated that there was no significant effect of thymectomy, X-irradiation or infection on the death rate recorded in the Tx-X group.

Tuberculin reaction. The reaction elicited by tuberculin 48 hours after injection into the wattle is presented in Table 4. Eighty-two percent of the Tx-X and all of the Tx chickens showed various degrees of response to tuberculin. Thymectomy did not appear to have any significant effect on the degree of tuberculin reaction in Tx and Tx-X chickens. One control infected chicken, which represented only 11% of this group, did not have any detectable reaction, even though gross and microscopic evidence of tuberculosis was noted in the liver and spleen. All noninfected chickens had no response to tuberculin when examined at 48 hours.

Thymectomy. The results of thymectomy are presented in Table 5. In the Tx-X group, 67% and 83% of the infected and uninfected chickens, respectively, did not have any thymic residua grossly. However, on microscopic examination of the thyroid and tissues in the adjacent areas, only 17% of each of

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Table 3. Three-way analysis of variance (nonreplicate) of the percent of chickens living at the end of the experiment

Source	df	SS	MS	F
Thymectomy	1	57.13805	57.13805	0.2659*
X-irradiation	1	6.09005	6.09005	0.0283*
Infection	1	240.46245	240.46245	1.1192*
Error	4	859.39545	214.84886	
Total	7	1163.08600		

*Not significant at 0.10 level.

Abbreviations: Source = source of variation; df = degrees of freedom; SS = sums of squares; MS = mean squares; F = $\frac{S_1^2}{S_2^2}$ where S_1^2 is the variance of sample size n_1 and S_2^2 is the variance of sample size n_2.

Table 4. Tuberculin reaction 48 hours after injection in chickens infected with *M. avium*

	Positive %	Negative %	Questionable %
Thymectomized	100	0	0
Thymectomized X-irradiated	82	9	9
Control	89	11	0
X-irradiated	86	0	14

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Table 5. Percentage of thymectomized chickens in which thymectomy was complete

		Tx		Tx-X	
		Gross	Microscopic	Gross	Microscopic
M. avium experiment	Infected	80*	0**	67	17
	Control	67	33	83	17
Group III experiment	Infected	88	38	43	14
	Control	75	0	75	50
M. bovis experiment	Infected	33	0	50	17
	Control	75	0	25	0

*Percent of total numbers of Tx chickens

**Percent of total numbers of Tx chickens

Abbreviations: Tx = thymectomized; Tx-X = thymectomized X-irradiated

the groups appeared to be completely devoid of thymic residua. Thymic remnants were detected close to, and sometimes posterior to, the thyroid gland. X-irradiation following thymectomy did not seem to have any effect on the thymic remnants as determined either grossly or microscopically. In the Tx infected group, none of the chickens was completely thymectomized. Thymectomy was complete in only 33% of the Tx uninfected chickens.

The last lobe of the thymus was found to be located very close to the thyroid gland on both sides. Instead of ending at the anterior pole of the thyroid, it sometimes extended over the gland for variable distances towards the posterior end. Quite frequently it reached the posterior end of the gland and was located as an accessory lobe between the thyroid and the

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the parathyroid gland (Figure 3). In other cases, although accessory lobes were present, their continuation with the last lobe of the thymus could not be demonstrated. Microscopically, these accessory lobes were characteristic of thymic tissue in all respects. Occasionally the thymus extended over the thyroid and attached closely to the latter as a thin, flat band (Figure 4).

In some cases, the thymic tissue was in intimate contact with the thyroid follicles, and there was no line of demarcation between these 2 structures (Figure 5). In some cases this could be demonstrated only with serial sections.

Skin at the site of inoculation. Raised nodular lesions of various sizes and forms were present at the sites of inoculation in all infected chickens. They varied from very small, grossly undetectable lesions to lesions of approximately 30 mm. in diameter. Most of the lesions were covered by thick, keratinized material, whereas others had ulceration and necrosis with central depression. As seen in Table 6, there were comparatively more small lesions (1 to 5 mm. in diameter) in the Tx-X group than in the other 3 groups. Thymectomy alone did not seem to have any marked effect on the size of the skin lesions.

Microscopically, varying quantities of ulceration and necrosis were seen in the skin lesions. Numerous granulomas with or without caseation were present. Histiocytes, giant cells, lymphocytes, plasma cells and acid-fast bacilli were present in variable numbers (Figure 6). These lesions at the sites of inoculation did not appear to be any different microscopically in Tx and Tx-X chickens than from those of the control and X-irradiated groups.

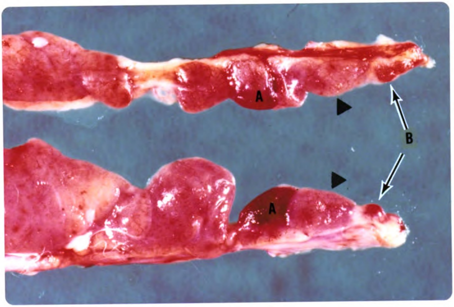

Figure 3. Thymic lobes from 10-week-old chicken. Note the accessory thymic lobe (arrows) between the thyroid (A) and the parathyroid (B) glands on either side.

Figure 4. Note a thin flat band of thymic tissue (arrows) around the thyroid gland (2). Hematoxylin and eosin. x 14.

Figure 5. Thymic tissue (1) in continuation with the thyroid gland (2). There is no line of demarcation between these 2 structures. Hematoxylin and eosin. x 210.

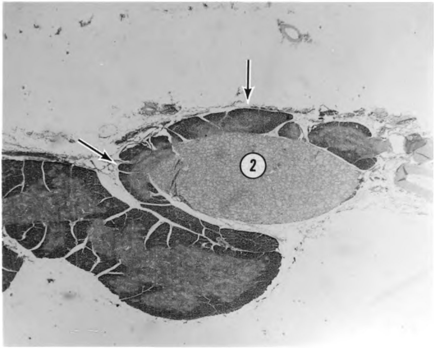

Figure 4

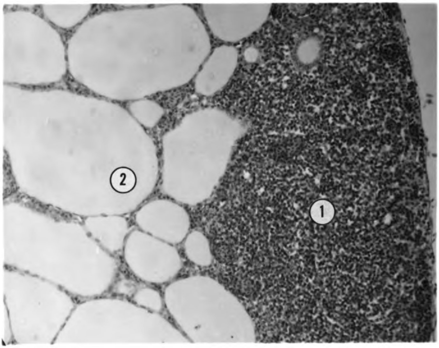

Figure 5

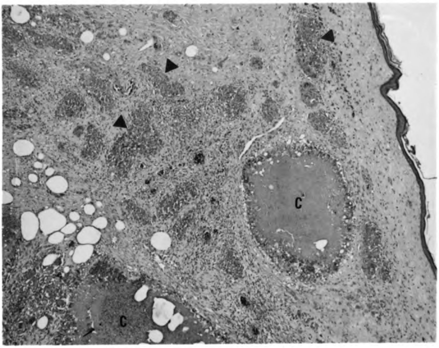

Figure 6. Skin inoculation site from a Tx-X chicken infected with *M. avium*. Note several noncaseous (arrows) and 2 caseous granulomas (C) in the dermis. New Fuchsin-Hematoxylin and eosin. x 59.

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Table 6. Diameters of the cutaneous lesions at the sites of inoculation

	1-5 mm. %	6-10 mm. %	11-15 mm. %	16-20 mm. %	20-30 mm. %
Thymectomized	0	60.0	40.0	0	0
Thymectomized X-irradiated	33.3	33.3	33.3	0	0
Control	12.5	62.5	25.0	0	0
X-irradiated	6.7	53.3	33.3	0	6.7

Lymphocytic cell distribution

I. Spleen. In the present study, various degrees of lymphocytic depletion were noted in the spleens of Tx and Tx-X chickens as compared to the large concentration of lymphocytes in the spleens of control and X-irradiated chickens (Figure 7). Of 12 Tx-X chickens infected with *M. avium*, only 4 had marked depletion of lymphocytic elements from the spleen (Figure 8). Three chickens from this group had moderate depletion and the other 5 chickens had no significant reduction of small lymphocytes.

Of 6 Tx-X chickens used in the uninfected group, slight and moderate amounts of lymphocytic depletion were noted in 2 and 3 chickens, respectively. One chicken did not have any appreciable reduction in the number of small lymphocytes.

Eight Tx chickens were used in this experiment. Five were infected with *M. avium* and 3 were not infected. Among the infected group, only 1 had evidence of lymphocytic depletion in the spleen. Two uninfected chickens had slight and moderate degrees of lymphocytic depletion, respectively. The third chicken did not have any appreciable reduction in small

Figure 7. Spleen from an uninfected control (nonthymectomized, non X-irradiated) chicken. Note the dense concentration of lymphocytes. New Fuchsin-Hematoxylin and eosin. x 69.

Figure 8. Spleen from a Tx-X chicken. Note marked depletion of lymphocytes. The lymphocytic nodules (arrows) are not affected. The dark staining areas represent red blood cell nuclei. New Fuchsin-Hematoxylin and eosin. x 69.

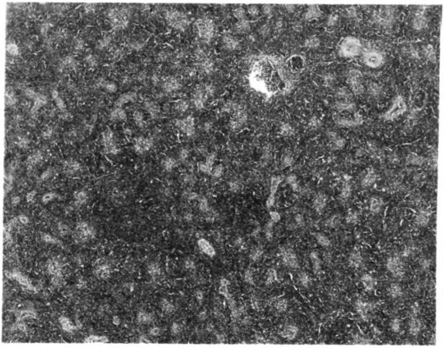

Figure 7

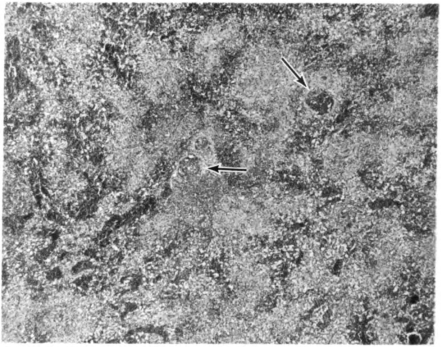

Figure 8

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lymphocytes. However, even in cases with severe lymphocytic depletion, no spleen was completely devoid of lymphocytes.

The bursa-dependent lymphocytic nodules in the splenic sections remained unaffected, although their numbers varied considerably.

Five of the splenic sections from 15 X-irradiated, infected chickens had marked lymphocytic depletion. Only 1 chicken from the control, infected group had reduced numbers of small lymphocytes in the spleen. In all of these chickens the depletion was generalized and was more evident around the Schweigger-Seidel sheaths (Figures 9 and 10). This type of change was due to amyloid deposition around the arteries. One spleen from an X-irradiated chicken had some lymphocytic depletion but no amyloidosis was evident.

II. Intestine. There was no noticeable reduction of lymphocytes in the sections taken at the ileocecal junction of Tx and Tx-X chickens.

III. Liver. Lymphocytic nodules (Figure 11) and small lymphocytes were present in variable numbers in the hepatic sections of chickens from all groups.

<u>Gross lesions</u>. The gross lesions observed at the time of necropsy are presented in Tables 7 and 8. Noninfected chickens did not have any gross lesions. The lesions described herein pertain only to infected chickens. Grossly, grayish-white focal lesions of various sizes were present. These were primarily in the liver and spleen. In some chickens hepatomegaly was evident along with numerous focal lesions. The size of the hepatic and splenic lesions varied from less than 1 mm. to a few millimeters in diameter. Although several chickens had hepatic lesions that appeared to be less than or close to 0.5 mm. in diameter, for convenience they were classified to be less than 1 mm. in diameter.

Figure 9. Spleen from an X-irradiated chicken infected with *M. avium*. Note lymphocytic depletion due to diffuse amyloidosis. New Fuchsin-Hematoxylin and eosin. x 69.

Figure 10. Spleen from an X-irradiated chicken infected with *M. avium*. Higher magnification of Figure 9. Note lymphocytic depletion due to amyloidosis around Schweigger-Seidel sheaths (1). Also note multinucleated giant cells (arrows). New Fuchsin-Hematoxylin and eosin. x 185.

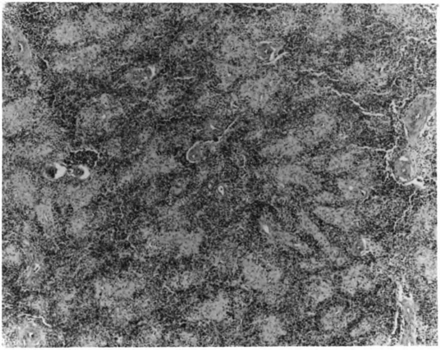

Figure 9

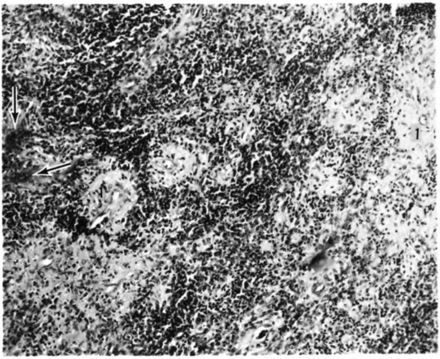

Figure 10

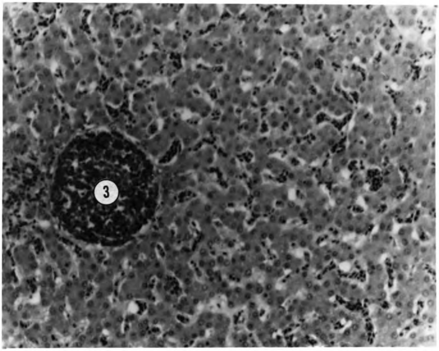

Figure 11. Lymphocytic nodule (3) in the liver of an X-irradiated chicken. New Fuchsin-Hematoxylin and eosin. x 250.

Table 7. Gross lesions and tuberculin reactions in Tx-X and Tx chickens infected with *M. avium*

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Group	Chicken No.	Hepatomegaly	Focal Gross Lesions				Thymectomy	Tuberculin reaction at 48 hours
			Liver	Spleen	Intestine	Lungs		
Thymectomized X-irradiated	308	-	+++	++	-	-	Complete	Bilateral swelling
	316	-	+	+	-	-	Complete	+
	317	-	++	++	-	-	Incomplete	+++
	319	-	+++	+++	-	-	Complete	+
	320	-	+++	++	-	-	Complete	Not recorded
	326	-	++	+	-	-	Complete	++++
	328	-	+	-	-	-	Incomplete	±
	330	-	+	+	-	-	Incomplete	Negative
	332	-	-	+	-	-	Complete	+++
	333	-	+++	++	+	-	Incomplete	Bilateral swelling
	340	-	+	+	-	-	Complete	+++
	342	+	+++	++	-	-	Complete	++
Thymectomized	302	-	+++	++++	-	-	Complete	+++
	304	+	+++	++	-	-	Complete	+++
	306	-	+++	++++	-	-	Incomplete	++
	307	-	+	-	-	-	Complete	++
	323	-	+++	+	-	-	Complete	+

Abbreviations: Gross lesions (liver and spleen)

+ = A few lesions - 1 mm. or less
++ = Moderate numbers of lesions - 1 mm. or less
+++ = Numerous lesions - 1 mm. or less
++++ = A few lesions > 1 mm. in diameter
+++++ = Moderate numbers of lesions > 1 mm. in diameter
++++++ = Large numbers of lesions > 1 mm. in diameter
- = No gross lesion

Tuberculin reaction

± = Questionable local swelling; + = Slight local swelling; ++ = Moderate swelling; +++ = Large swelling; ++++ = Large swelling involving most of the wattle; Bilateral swelling = swelling of both wattles. This was often seen in pullets since they usually have small wattles.

Table 8. Gross lesions and tuberculin reactions in X-irradiated and control chickens infected with *M. avium*

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Group	Chicken No.	Hepatomegaly	Focal Gross Lesions					Tuberculin reaction at 48 hours
			Liver	Spleen	Intestine	Lungs	Thymus	
X-irradiated	303	-	+	-	-	-	-	±
	305	-	+	+	-	-	-	++++
	310	+	+++	++++++	-	+	-	Bilateral swelling
	314	-	+	++	-	-	-	+
	315	-	++	++++	-	-	-	++
	321	-	+++	++++	-	-	-	Not recorded
	322	-	++	++	-	-	-	+
	324	+	+++	+++	-	-	+	++++
	327	+	+++	+++	-	-	-	±
	329	-	+++	+	-	-	-	+++
	331	-	+++	+	-	-	-	++ (bilateral)
	334	+	++++++	++	-	-	-	Bilateral swelling
	336	-	+	+	-	-	+	++
	339	-	+++	++	-	-	-	Bilateral swelling
	341	+	++++++	+++++	-	-	-	++
Control	309	+	++++++	+++++	+	+	-	Bilateral swelling
	311	-	+++++	++	-	-	-	+
	312	-	+++	+++	-	-	-	++

Table 8 (cont'd.)

Table 8 (cont'd.)

Group	Chicken No.	Hepatomegaly	Focal Gross Lesions					Tuberculin reaction at 48 hours
			Liver	Spleen	Intestine	Lungs	Thymus	
Control (cont'd.)	313	-	+	+	-	-	-	++
	318	+	++++++	+++++	-	-	-	+
	325	-	+++++	++++	-	-	-	++++
	335	-	+	++++	-	-	-	Negative
	337	-	++	++++	-	+ (on air sac)	-	++
	338	-	-	++	-	-	-	++

Abbreviations: Gross lesions (liver and spleen)

+ = A few lesions - 1 mm. or less
++ = Moderate numbers of lesions - 1 mm. or less
+++ = Numerous lesions - 1 mm. or less
++++ = A few lesions > 1 mm. in diameter
+++++ = Moderate numbers of lesions > 1 mm. in diameter
++++++ = Large numbers of lesions > 1 mm. in diameter
- = No gross lesion

Tuberculin reaction

± = Questionable local swelling
+ = Slight local swelling
++ = Moderate swelling
+++ = Large swelling
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Bilateral = swelling of both the wattles. This was often seen in pullets since they usually have small wattles.

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Of 9 control infected chickens, only 1 did not have any grossly detectable lesions in the liver. All chickens in this group had varying numbers of lesions in the spleen. One chicken from a group of 15 X-irradiated chickens did not have any lesion in the spleen. All chickens in this group had gross lesions in the liver. Gross lesions were also present in the livers of all Tx chickens. Only 1 chicken (20%) from this group did not have any gross lesions in the spleen. Hepatic and splenic granulomas were absent in 1 of 12 Tx-X chickens.

Small grayish-white lesions were also present at the ileocecal junction of 2 chickens (1 control and 1 Tx); in the lungs of 2 chickens (1 X-irradiated and another control); and in the thymus glands of 2 X-irradiated chickens.

Microscopic lesions. Granulomas, with or without caseation, were present in the liver, spleen and, occasionally, in the ileocecal junction, lungs

Figure 12. Liver and spleen from a control chicken infected with *M. avium*. Note numerous circumscribed grayish-white lesions.

Figure 13. Liver and spleen from an X-irradiated chicken infected with *M. avium*. Note numerous grayish-white sharply demarcated lesions.

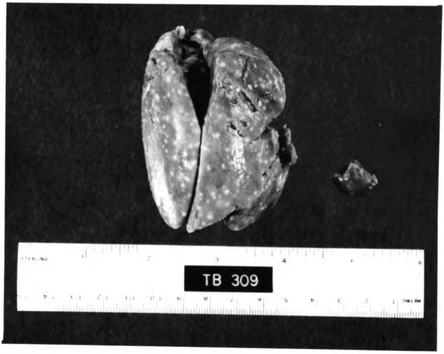

Figure 12

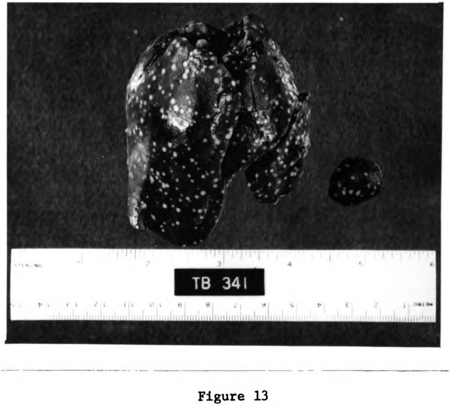

Figure 13

Figure 14. Liver from a Tx chicken infected with *M. avium*. Note numerous circumscribed pin-point lesions.

Figure 15. Liver from a Tx-X chicken infected with *M. avium*. Note numerous grayish-white circumscribed pin-point lesions.

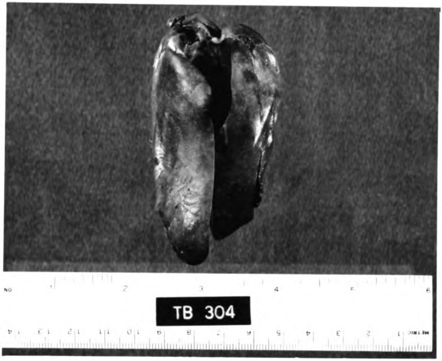

Figure 14

Figure 15

[illegible] reactions in Tx-X and Tx chickens infected with *M. avium*

Table 7. Gross lesions and tuberculin reactions in Tx-X and Tx chickens infected with *M. avium*

Group	Chicken No.	Hepatomegaly	Focal Gross Lesions Liver	Spleen	Intestine	Lungs	Thymectomy	Tuberculin reaction at 48 hours
Thymectomized X-irradiated	308	-	+++	++	-	-	Complete	Bilateral swelling
	316	-	+	+	-	-	Complete	+
	317	-	++	++	-	-	Incomplete	+++
	319	-	+++	+++	-	-	Complete	+
	320	-	+++	++	-	-	Complete	Not recorded
	326	-	++	+	-	-	Complete	++++
	328	-	+	-	-	-	Incomplete	±
	330	-	+	+	-	-	Incomplete	Negative
	332	-	-	+	-	-	Complete	+++
	333	-	+++	++	+	-	Incomplete	Bilateral swelling
	340	-	+	+	-	-	Complete	+++
	342	+	+++	++	-	-	Complete	++
Thymectomized	302	-	+++	++++	-	-	Complete	+++
	304	+	+++	++	-	-	Complete	+++
	306	-	+++	++++	-	-	Incomplete	++
	307	-	+	-	-	-	Complete	++
	323	-	+++	+	-	-	Complete	+

Abbreviations: Gross lesions (liver and spleen)

+ = A few lesions - 1 mm. or less
++ = Moderate numbers of lesions - 1 mm. or less
+++ = Numerous lesions - 1 mm. or less
++++ = A few lesions > 1 mm. in diameter
+++++ = Moderate numbers of lesions > 1 mm. in diameter
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\- = No gross lesion

Tuberculin reaction

± = Questionable local swelling; + = Slight local swelling; ++ = Moderate swelling; +++ = Large swelling; ++++ = Large swelling involving most of the wattle; Bilateral swelling = swelling of both wattles. This was often seen in pullets since they usually have small wattles.

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Group	Chicken No.	Hepatomegaly	Focal Gross Lesions					Tuberculin reaction at 48 hours
			Liver	Spleen	Intestine	Lungs	Thymus	
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	305	-	+	+	-	-	-	++++
	310	+	+++	++++++	-	+	-	Bilateral swelling
	314	-	+	++	-	-	-	+
	315	-	++	++++	-	-	-	++
	321	-	+++	++++	-	-	-	Not recorded
	322	-	++	++	-	-	-	+
	324	+	+++	+++	-	-	+	++++
	327	+	+++	+++	-	-	-	±
	329	-	+++	+	-	-	-	+++
	331	-	+++	+	-	-	-	++ (bilateral)
	334	+	++++++	++	-	-	-	Bilateral swelling
	336	-	+	+	-	-	+	++
	339	-	+++	++	-	-	-	Bilateral swelling
	341	+	++++++	+++++	-	-	-	++
Control	309	+	++++++	+++++	+	+	-	Bilateral swelling
	311	-	+++++	++	-	-	-	+
	312	-	+++	+++	-	-	-	++

Table 8 (cont'd.)

Group	Chicken No.	Hepatomegaly	Focal Gross Lesions Liver	Spleen	Intestine	Lungs	Thymus	Tuberculin reaction at 48 hours
Control (cont'd.)	313	-	+	+	-	-	-	++
	318	+	++++++	+++++	-	-	-	+
	325	-	+++++	++++	-	-	-	++++
	335	-	+	++++	-	-	-	Negative
	337	-	++	++++	-	+ (on air sac)	-	++
	338	-	-	++	-	-	-	++

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Small grayish-white lesions were also present at the ileocecal junction of 2 chickens (1 control and 1 Tx); in the lungs of 2 chickens (1 X-irradiated and another control); and in the thymus glands of 2 X-irradiated chickens.

<u>Microscopic lesions</u>. Granulomas, with or without caseation, were present in the liver, spleen and, occasionally, in the ileocecal junction, lungs

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Figure 13. Liver and spleen from an X-irradiated chicken infected with *M. avium*. Note numerous grayish-white sharply demarcated lesions.

Figure 12

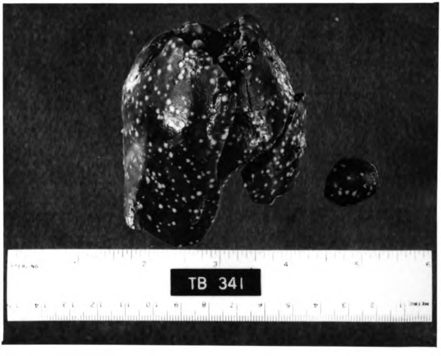

Figure 13

Figure 14. Liver from a Tx chicken infected with *M. avium*. Note numerous circumscribed pin-point lesions.

Figure 15. Liver from a Tx-X chicken infected with *M. avium*. Note numerous grayish-white circumscribed pin-point lesions.

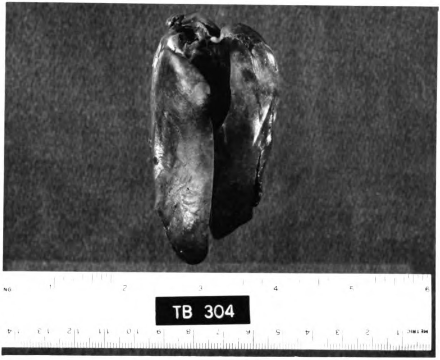

Figure 14

Figure 15

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and thymus glands of chickens from all infected groups. Histologically, granulomas of the following stages of development were encountered:

1. Granulomas consisting of epithelioid cells, histiocytes, heterophils and plasma cells (Figure 16).

2. Granulomas with early central necrosis (Figure 17).

3. Granulomas with a small quantity of necrosis in the center. The cellular infiltration was similar to that of stage 1 (Figure 18).

4. Granulomas with large areas of necrosis in the center immediately surrounded by giant cells. The outer zone was composed of histiocytes, granulocytes, fibroblasts and plasma cells (Figure 19).

Lymphocytes were present in varying numbers. Their presence in the tubercles was inconsistent. Sometimes they accumulated in thin layers at the periphery of the lesion (Figure 20).

In noncaseous lesions, acid-fast bacilli were usually seen in small numbers in the cytoplasm of histiocytes. Quite frequently acid-fast organisms were seen in the center of caseous granulomas. Although their numbers varied, the organisms were more numerous in noncaseous lesions. In some lesions large aggregates of bacilli were noted (Figure 21). There was no difference in the number of acid-fast organisms present in the lesions of Tx and Tx-X chickens compared with control and X-irradiated chickens. However, since more caseous lesions were noted in the organs of control and X-irradiated chickens, and caseous lesions appeared to contain more organisms than noncaseous ones, it may be that the total numbers of bacilli present in control and X-irradiated chickens may have been larger than in those of the Tx and Tx-X chickens. Amyloidosis was present in varying degrees in and around many tubercles in the liver and spleen. The deposition was more prominent in the spleen than in the liver. In the spleen 2 different patterns of amyloidosis were evident. In some spleens

Figure 16. First stage granuloma in the liver of *M. avium*-infected chicken consisting of histiocytes, heterophils and plasma cells. New Fuchsin-Hematoxylin and eosin. x 230.

Figure 17. Second stage granuloma in the liver of *M. avium*-infected chicken with early necrosis in the center (arrow). Note a thin connective tissue capsule around the lesion. New Fuchsin-Hematoxylin and eosin. x 151.

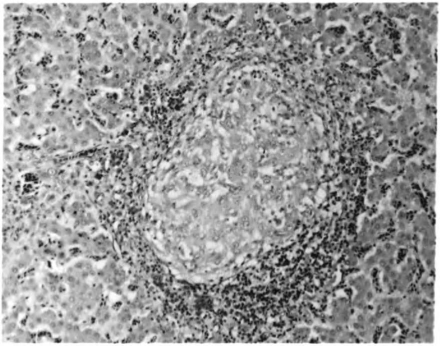

Figure 16

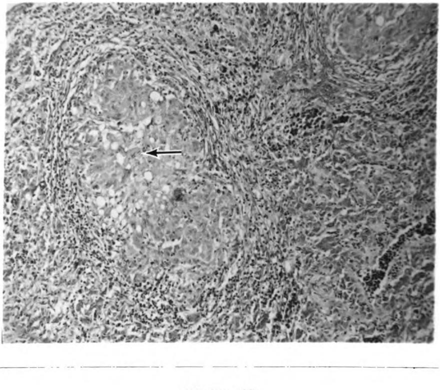

Figure 17

Figure 18. Third stage granuloma in the liver of *M. avium*-infected chicken with small amount of central necrosis (arrow). New Fuchsin-Hematoxylin and eosin. x 97.

Figure 19. Fourth stage granuloma in the liver of *M. avium*-infected chicken with large amounts of necrosis (3). New Fuchsin-Hematoxylin and eosin. x 71.

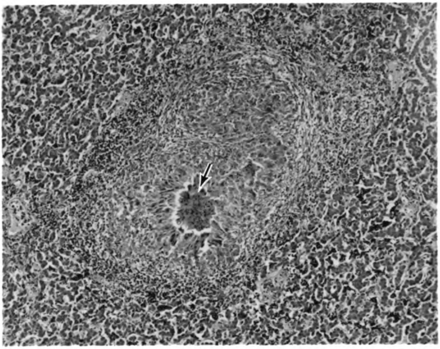

Figure 18

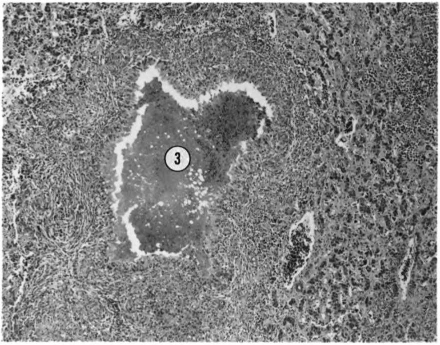

Figure 19

Figure 20. Granuloma in the liver of an X-irradiated chicken infected with *M. avium*. Note an outer zone of lymphocytes (arrows). New Fuchsin-Hematoxylin and eosin. x 157.

Figure 21. Fourth stage granuloma in the spleen of a control chicken infected with *M. avium*. Note clumps of acid-fast organisms in the necrotic tissue (arrow). New Fuchsin-Hematoxylin and eosin. x 200.

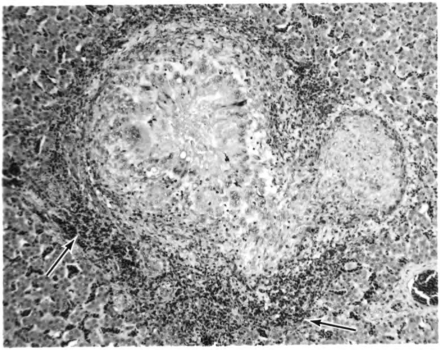

Figure 20

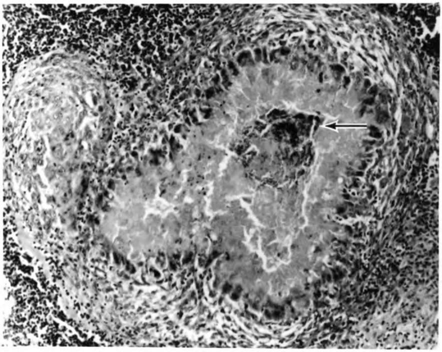

Figure 21

the amyloid was deposited in the form of dense, homogeneous material in and around the tubercles (Figure 22). In other cases there was diffuse periarterial amyloidosis (Figures 9 and 10) involving the entire spleen. This type of change was more pronounced around the Schweigger-Seidel sheaths of the spleen. Among the infected chickens, this type of reaction was noted in 11% of control, 33% of X-irradiated, 20% of Tx and in 17% of the Tx-X chickens. Varying quantities of lymphocytic tissue necrosis were evident in spleens with diffuse amyloidosis.

In the liver, amyloid was seen in the sinusoids, around portal veins (Figure 23) and in the tubercles. The incidence of amyloidosis in the spleen and liver of *M. avium*-infected chickens is shown in Table 9.

In 2 splenic sections with diffuse amyloidosis, giant cells were seen in the area of amyloid deposit (Figure 10). A few granulomas had some mineralization at the center of the necrotic tissue (Figure 24).

I. Liver. Some hepatic sections from infected chickens had varying degrees of necrosis and disappearance of hepatocytes. Cells of the granulocytic series were present in large numbers around portal areas and elsewhere in some sections. Occasionally cells with large, vesicular nuclei were also seen in large numbers. They probably represented immature cells of the granulocytic series. Some hepatic sections had increased numbers of plasma cells.

Tubercles of all 4 stages were present in the liver. However, the number of tubercles of the first stage appeared to be larger in this organ than in the spleen. The total number of tubercles were counted from each hepatic section. The percentages of lesions that had central necrosis are given in Table 10.

Figure 22. Tubercle in the spleen of an X-irradiated chicken infected with *M. avium*. Note dense homogeneous material around the tubercle (arrows). New Fuchsin-Hematoxylin and eosin. x 104.

Figure 23. Liver from an X-irradiated chicken infected with *M. avium*. Note diffuse amyloidosis immediately surrounding the portal vein (4). New Fuchsin-Hematoxylin and eosin. x 59.

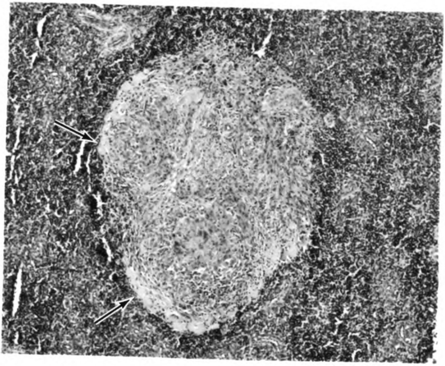

Figure 22

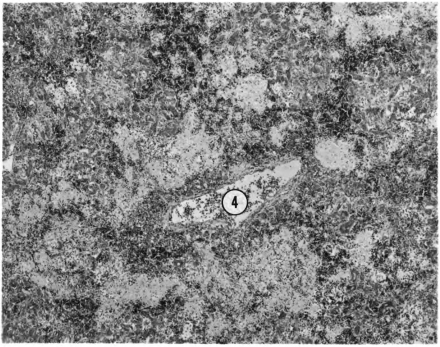

Figure 23

Figure 22. Tubercle in the spleen of an X-irradiated chicken infected with *M. avium*. Note dense homogeneous material around the tubercle (arrows). New Fuchsin-Hematoxylin and eosin. x 104.

Figure 23. Liver from an X-irradiated chicken infected with *M. avium*. Note diffuse amyloidosis immediately surrounding the portal vein (4). New Fuchsin-Hematoxylin and eosin. x 59.

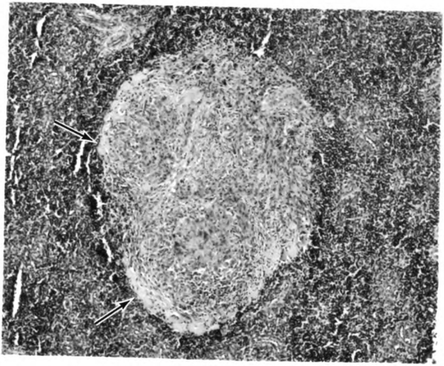

Figure 22

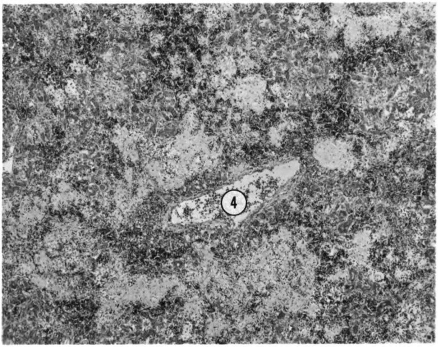

Figure 23

Table 9. Incidence of amyloidosis in the liver and spleen of *M. avium*-infected chickens

	Tx %	Tx-X %	C %	X %
Liver alone	0	17	22	0
Spleen alone	60	25	56	47
Both liver and spleen	20	33	11	33

Abbreviations: Tx = thymectomized; Tx-X = thymectomized X-irradiated; C = control (nonthymectomized, non X-irradiated); X = X-irradiated.

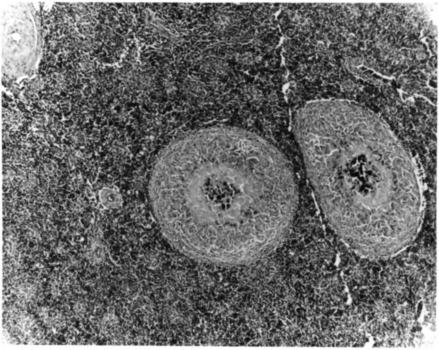

Figure 24. Spleen from a Tx chicken infected with *M. avium*. Note mineralization in the center of the tubercles. New Fuchsin-Hematoxylin and eosin. x 69.

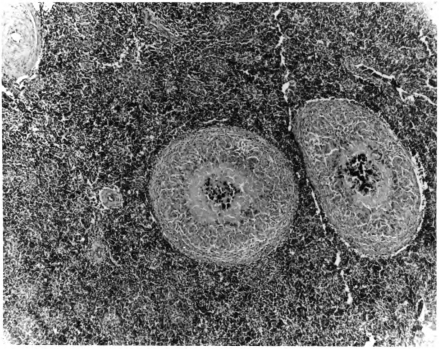

Figure 24. Spleen from a Tx chicken infected with *M. avium*. Note mineralization in the center of the tubercles. New Fuchsin-Hematoxylin and eosin. x 69.

Table 10. Percentage of caseous granulomas in *M. avium*-infected chickens

	Tx	Tx-X	C	X
Liver	9.7	15.9	25.5	30.00
Spleen	24.8	30.7	38.9	42.5

Abbreviations: Tx = thymectomized; Tx-X = thymectomized X-irradiated; C = control (nonthymectomized, non X-irradiated); X = X-irradiated.

In the Tx and Tx-X chickens, the percentage of caseous granulomas appeared to be lower than in the other 2 groups. Caseous granulomas from the Tx and Tx-X birds often had minimal amounts of tissue necrosis. However, chicken No. 333 from the Tx-X group had unusually large numbers of caseous granulomas (Table 11). These data were further subjected to statistical analysis to see if the smaller numbers of caseous granulomas in the livers of Tx and Tx-X chickens were significant. All data were transformed by using the formula $Y' = \sqrt{Y+1}$. The results are presented in Table 12. The Tx and Tx-X chickens had significantly fewer caseous lesions in the liver ($\alpha = 0.10$).

II. Spleen. Caseous and noncaseous granulomas were present in varying numbers in the splenic sections. These lesions were counted and are presented in Table 11. In some sections there was considerable overlapping of lesions which made it difficult to determine accurate counts. As can be seen from the table, in the spleen there appeared to be a greater number of lesions with caseous centers than in the liver. However, the number of noncaseous granulomas was still higher than the number of caseous lesions. It is evident from Table 10 that the percentage of caseous

Table 11. Numbers of caseous and noncaseous lesions in the hepatic and splenic sections of *M. avium*-infected chickens

Group	Chicken No.	Liver		Spleen	
		Caseous	Noncaseous	Caseous	Noncaseous
Thymectomized X-irradiated	308	1	3	2	3
	316	0	5	1	13
	317	2	6	8	17
	319	1	10	4	7
	320	1	25	7	10
	326	0	6	3	5
	328	0	0	0	0
	330	0	1	6	3
	332	0	1	0	2
	333	9	5	9	18
	340	0	1	3	8
	342	1	16	9	31
Thymectomized	302	2	6	4	15
	304	4	29	18	48
	306	0	3	3	5
	307	0	1	0	1
	323	0	17	4	19
X-irradiated	303	0	1	0	1
	305	4	9	4	3
	310	10	30	39	43
	314	1	1	7	2
	315	1	5	4	28
	321	1	6	5	6
	322	5	1	6	10
	324	0	32	13	35
	327	3	13	3	8
	329	1	4	2	7
	331	13	18	15	19
	334	3	6	2	1
	336	0	4	0	2
	339	2	14	6	8
	341	6	2	9	7
Control	309	11	15	15	13
	311	3	4	13	16
	312	0	6	26	36
	313	0	0	1	4
	318	5	15	9	15
	325	5	14	4	9
	335	0	5	25	47
	337	3	4	12	6
	338	0	0	14	15

Table 12. Two-way analysis of variance (nonreplicate) of the caseation necrosis in hepatic lesions of *M. avium*-infected chickens

	Source	df	SS	MS	F
A.	Thymectomy	1	2.01437	2.01437	3.4670*
B.	X-irradiation	1	0.01608	0.01608	0.0277
	AXB interaction	1	0.02866	0.02866	0.0493
	Error	37	21.49726	0.58101	
	Total	40	23.55637		

*Significant at 0.10 level

Abbreviations: Source = source of variation; df = degrees of freedom; SS = sums of squares; MS = mean squares; F = $\frac{S_1^2}{S_2^2}$ where S_1^2 is the variance of sample size n_1 and S_2^2 is the variance of sample size n_2

lesions in the spleen was comparatively less in Tx and Tx-X chickens than in the X-irradiated and control chickens. Also the splenic lesions in the former 2 groups were somewhat smaller than those from the control and X-irradiated chickens, although individual variations did occur. Statistical evaluation of the data did not reveal any significant effect of thymectomy or X-irradiation on the percentage of caseous or noncaseous lesions produced in the spleen of infected chickens. These data were further analyzed by a nonparametric sign test (Siegel, 1956). In the Tx-X chickens, there were significantly more noncaseous lesions than caseous lesions ($\alpha = 0.05$). In the other 3 groups, there was no significant difference between the incidence of caseous and noncaseous granulomas.

III. Intestine. Caseous and noncaseous granulomas of various sizes were noted at the ileocecal junction of several chickens (Figure 25).

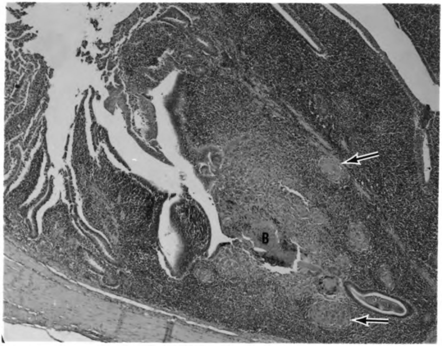

Figure 25. Ileocecal junction of a control chicken infected with *M. avium*. Note caseous (B) and noncaseous (arrows) granulomas in the lamina propria. New Fuchsin-Hematoxylin and eosin. x 59.

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Varying numbers of acid-fast bacilli were seen in these lesions. The granulomas were present in 67% of Tx-X, 47% of X-irradiated, and 22% of control chickens. Granulomas were not seen at the ileocecal junction of Tx chickens.

IV. Thymus. Caseous and noncaseous granulomas of various sizes were present in the thymic remnants of 58% of Tx-X and 40% of Tx chickens. Approximately 33% of control and 59% of X-irradiated chickens had granulomas in their thymus glands (Figure 26).

V. Lungs. Granulomas, with or without caseation, were noted in the lungs of 25% of Tx-X, 25% of Tx, 33% of X-irradiated and 38% of control chickens. Varying numbers of acid-fast organisms were noted in these lesions.

Bacteriologic findings. Acid-fast organisms were isolated from the pools of livers and spleens of all the chickens infected with *M. avium*. Organisms were not isolated from any of the noninfected chickens.

Group III Experiment

Fifty-three chickens were utilized in this experiment (Table 1). Of this number, 35 were infected with a Group III mycobacterium of bovine origin and the remaining 18 chickens were not infected. All the chickens survived until the end of the experiment.

Tuberculin reaction. The reaction elicited by tuberculin 48 hours after injection into the wattle is recorded in Table 13.

Thymectomy. The results of thymectomy are presented in Table 5. As shown, some chickens that appeared free of thymic residua grossly did have some thymic remnant when examined microscopically.

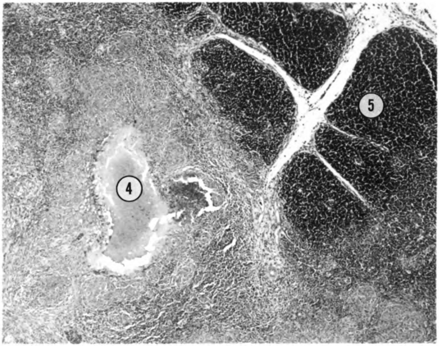

Figure 26. Thymus of an X-irradiated chicken infected with *M. avium*. Note 1 large caseous granuloma (4) occupying the medulla. The cortex (5) is unaffected. New Fuchsin-Hematoxylin and eosin. x 70.

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Table 13. Tuberculin reaction 48 hours after injection in chickens infected with Group III mycobacterium

	Positive %	Negative %	Questionable %
Thymectomized	100	0	0
Thymectomized X-irradiated	86	14	0
Control	30	70	0
X-irradiated	90	10	0

Skin at the site of inoculation. During gross examination, small scars were noted at the inoculation sites in 50% of control, 60% of X-irradiated, 63% of Tx and 57% of Tx-X chickens. No correlation could be established between the presence of the scar at the site of inoculation and the type of treatment the chickens received.

Histologically, varying numbers of tubercles consisting of histiocytes were noted at the skin sites. The lesions were located in the dermis and were present in almost all chickens that had visible scars at the site of inoculation. A few granulomas with caseous centers were also seen at some inoculation sites (Figure 27). Acid-fast organisms were noted in most of the skin lesions. Lymphocytes and plasma cells were present in variable numbers. Perivascular cuffing with lymphocytes was commonly seen. Some chickens which did not have any detectable lesions at the skin site during gross examination had small tubercles upon microscopic evaluation.

Lymphocytic cell distribution

I. Spleen. In the infected group, 75% of Tx and 29% of Tx-X chickens did not have any lymphocytic depletion from the white pulp of the spleen.

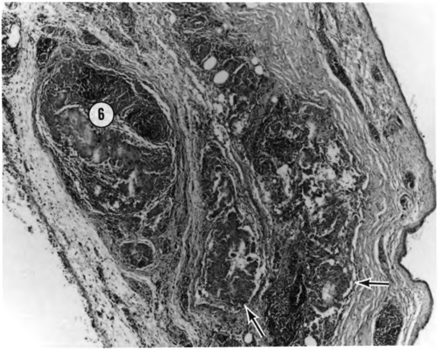

Figure 27. Skin inoculation site from an X-irradiated chicken infected with Group-III mycobacterium. Note the presence of caseous (6) and noncaseous (arrows) granulomas in the dermis. New Fuchsin-Hematoxylin and eosin. x 59.

Other chickens had various degrees of lymphocytic depletion from the spleen. Variable numbers of plasma cells were present in the splenic sections of chickens from all 4 groups. In the noninfected group, 50% of Tx and all Tx-X chickens had variable amounts of reduction in small lymphocytes from the splenic white pulp.

II. Intestine. There was no detectable reduction of lymphocytes from the sections of intestine taken at the level of the ileocecal junction.

Gross lesions. Gross lesions were not seen in the liver and spleen of any of the chickens except 1 Tx chicken, which had a few small white foci in the spleen. A few white foci were also noted in the yolk sac of a Tx chicken and on the peritoneum of a Tx-X chicken. One control uninfected chicken had 2 or 3 small white foci on one air sac.

Microscopic lesions. Microscopically, tuberculous granulomas were not seen in any tissue section. The spleen from chicken No. 449, which had a few white foci on gross examination, did not have any microscopic lesion. Microscopically, lesions were not seen in the yolk sac and in the air sac of chickens that had some evidence of gross lesions. A few small granulomas with mineralization were present in the peritoneum of chicken No. 447, which had gross lesions. In 2 other chickens, small granulomas were present in the ileocecal junction. However, acid-fast organisms were not seen in any of the aforementioned lesions.

Bacteriologic findings. Acid-fast organisms were isolated from the pools of livers and spleens of 25% of Tx infected, 29% of Tx-X infected, and 30% of control infected chickens. Organisms were not isolated from any of the X-irradiated infected chickens.

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Bacteriologic findings. Acid-fast organisms were isolated from the pools of livers and spleens of 25% of Tx infected, 29% of Tx-X infected, and 30% of control infected chickens. Organisms were not isolated from any of the X-irradiated infected chickens.

M. bovis Experiment

Forty-nine chickens belonging to the various treatment groups were used in this experiment (Table 1). Of this number, 31 were infected with a virulent strain of *M. bovis*. The remaining 18 chickens were not infected. All the chickens survived until the end of the experiment.

Tuberculin reaction. In this experiment 4 different readings of the tuberculin reaction site were taken beginning at 2 hours after inoculation. The results are recorded in Table 14. Approximately 84% of infected and 67% of noninfected chickens had some degree of swelling at 2 hours after injection. However, the swellings in all uninfected and some infected chickens were very slight and probably represented nonspecific local reaction to the injection of tuberculin. None of the uninfected chickens had detectable swelling at 48 hours. Some infected chickens that had detectable reaction at 48 hours also had variable degrees of reaction to tuberculin at 2 hours.

Thymectomy. The results of thymectomy are listed in Table 5. During gross examination of infected chickens, 33% of Tx and 50% of Tx-X chickens appeared to be completely thymectomized. However, on microscopic examination, all the Tx infected and 83% of the Tx-X infected chickens had some thymic tissue in the vicinity of the thyroid gland. Microscopically, none of the Tx uninfected or Tx-X uninfected chickens was completely thymectomized.

Skin at the site of inoculation. There were no detectable lesions at inoculation sites.

Lymphocytic cell distribution

I. Spleen. Varying amounts of lymphocytic depletion from the spleen were noted in 33% of the Tx infected and 50% of Tx uninfected chickens.

Table 14. Tuberculin reaction at various time intervals in *M. bovis*-infected and noninfected chickens

Group	Chicken No.	2 hours	5 hours	27 hours	48 hours
			Infected		
Thymectomized	512	++	++	+	-
	522	++	+	±	-
	527	±	-	-	-
	528	-	-	-	-
	530	±	+	-	-
	544	+	+	+	++
Thymectomized X-irradiated	506	++	++	±	±
	510	±	±	-	-
	515	±	-	-	-
	517	+	±	±	-
	531	-	-	-	-
	537	++	+	±	-
Control	508	+	-	-	-
	511	++	+	±	-
	516	++	++	++	+
	518	+	±	-	-
	519	++	±	-	-
	520	+	±	-	±
	521	++	++	++	+
	523	±	-	-	-
	524	-	-	-	-
	525	-	-	-	-
X-irradiated	507	+	±	-	-
	509	++	++	+++	++
	513	++	+	+	±
	514	+	++	+	-
	526	+	±	±	+
	529	+	+	+	+
	532	±	-	±	-
	533	+	+	-	-
	536	-	±	-	-
			Noninfected		
Thymectomized	546	-	-	-	-
	547	±	±	-	-
	551	±	±	-	-
	552	±	±	-	-

Table 14 (cont'd.)

Group	Chicken No.	2 hours	5 hours	27 hours	48 hours
Thymectomized X-irradiated	538	±	-	-	-
	540	-	-	-	-
	548	-	-	-	-
	549	-	-	-	-
Control	535	±	±	±	-
	541	±	-	-	-
	545	-	±	-	-
	550	-	-	-	-
	553	±	-	-	-
X-irradiated	534	±	±	-	-
	539	±	-	-	-
	542	±	-	±	-
	543	±	±	-	-
	554	±	-	-	-

Abbreviations: ± = questionable local swelling
+ = slight local swelling
++ = moderate swelling
+++ = large swelling
++++ = large swelling involving most of the wattle

Seventy percent of Tx-X uninfected chickens and none of the Tx-X infected chickens had lymphocytic depletion from the spleen.

II. Intestine. Sections of intestine taken at the ileocecal junction did not appear to have any depletion in the number of lymphocytes in any treatment group.

Gross lesions. Three infected chickens, one each belonging to the control, X-irradiated and Tx-X groups, had hepatic lesions characterized by 1 white spot on the liver. Gross lesions were not detectable in any other organ. One pin-point white focus was seen on the liver of an X-irradiated uninfected chicken.

Microscopic lesions. One control infected and another X-irradiated uninfected chicken, that showed gross lesions in the liver, did not have any microscopic lesion. One X-irradiated infected chicken with a gross lesion in the liver also had a microscopic lesion which was characterized by focal accumulation of lymphocytes and heterophils. Focal accumulation of lymphocytes and heterophils was also noted in the hepatic sections of 2 infected chickens belonging to the X-irradiated and Tx groups, respectively. There were no lesions noted during gross examination.

One infected Tx-X chicken, that had gross lesions in the liver, had microscopic evidence of a hepatic neoplasm. The neoplasm was lightly encapsulated and was composed of irregularly arranged hepatocytes which had moderately hyperchromatic nuclei. Mitotic figures were rare. This neoplasm was diagnosed as hepatoma of low grade malignancy.

Characteristic tuberculous granulomas were not seen in any of the tissues examined.

Bacteriologic findings. Acid-fast organisms were not isolated from any of the pooled samples.

Thymus-Thyroid Relationship

Thirty chickens, 10 each from various age groups, were examined to determine the relationship between the thyroid gland and the thymus. In several instances accessory thymic lobes of various sizes were seen grossly at the posterior end of the thyroid gland, either on the left or on the right side. This is presented in Table 15.

Table 15. Incidence of accessory thymic lobes in chickens of various ages

Age	No accessory lobes on either side	Accessory lobes	
		Right side	Left side
10 weeks	1*/10	4/10	6/10
5 weeks	2/10	6/10	4/10
1 day	2/10	3/10	5/10

*Numerator, no. of chickens with or without accessory lobes; denominator, no. of chickens examined.

In some chickens accessory lobes were present on both sides. In one case one ectopic lobe of the thymus was located on the ventral aspect of the esophagus.

The number of thymic lobes on each side varied from 5 to 7. Occasionally the thymic substance was composed of 4 lobes on one side. This variation in the number of lobes was due to the fusion of the adjacent lobes. Occasionally, the thyroid was covered by thin flaps of thymic substance.

DISCUSSION

Death rate after infection with *M. avium*. Approximately 30% of the Tx-X chickens infected with *M. avium* died before termination of the experiment. However, the death rate in this group was not significantly higher statistically than in the control and X-irradiated groups. Three Tx-X chickens of 5 that died had tuberculous granulomas and probably died from relatively acute tuberculosis. The cause of death in the other 2 chickens could not be determined. Higher death rate in Tx-X chickens has also been reported by other workers (Peterson *et al.*, 1964; Druet and Janigan, 1966).

Tuberculin reaction. All of the uninfected birds had no detectable reaction.

A high percentage of Tx-X and all Tx chickens infected with *M. avium* and Group-III mycobacterium had various degrees of response to tuberculin. Other infected chickens also had tuberculin reactions, but the tuberculin response of Tx-X chickens did not agree with the observation of Cooper *et al.* (1966a). These authors reported that of 14 chickens sensitized to diphtheria toxoid with complete Freund's adjuvant, only 2 had positive wattle reaction to the same antigen. In contrast, all the control chickens had positive wattle reaction beginning at 5 hours and reaching maximum at 24 hours. This observation led Cooper and associates to suggest that the thymus was responsible for the mediation of delayed hypersensitivity reactions. If this was true, then the Tx-X chickens in the present study should not have had any tuberculin response. However, the observations of Cooper *et al.* cannot be directly compared with the results of the present experiments because of the following reasons.

First, Cooper *et al.* did not report tuberculin response in their chickens at 48 hours, which was the only reading taken in the present investigation. It is possible that some of the chickens in their experiment that did not have positive response at 24 hours could have shown some reaction at 48 hours.

Second, in the work by Cooper and co-workers (1966a) the chickens were tested for delayed hypersensitivity only a week after sensitization. At this time none of the sensitized birds had detectable circulating antibody to diphtheria toxoid. Although the chickens in the present study were not tested for circulating antibody, it is possible that they had some antibody at the time of testing and that this was responsible for an Arthus reaction which persisted and was not differentiated from the delayed response. It is known that antibodies are produced in tuberculosis. Some antibodies may give rise to the Arthus reaction thereby complicating delayed reaction. Some evidence has been presented by Boyden (1964) in support of the fact that the lag period between sensitization and tuberculin testing does influence the tuberculin test results. Boyden sensitized guinea pigs to sheep red cells with complete Freund's adjuvant. When these sensitized animals were skin-tested soon after immunization, they showed classical delayed response. There was no detectable increase in skin thickness at 4 hours, but there were positive reactions at 24 and 48 hours. In contrast to this, when the animals were skin-tested at a later date, most of the reactions started at 4 hours and reached maximum at 48 hours. Boyden (1964) believed that the skin reactions in the latter group were complicated by Arthus reaction which appeared at 4 hours.

The Arthus reaction may be severe and persist for long periods. The Arthus reaction can generally be differentiated from the delayed response by the following means (Davis *et al.*, 1967).

I. The Arthus reaction starts around 2 hours after injection of the antigen and reaches maximum at 4 to 5 hours. By 24 hours most of the reaction has vanished.

Delayed reaction becomes visible approximately 10 to 12 hours after injection and reaches maximum at 24 to 48 hours.

II. Histologically, the Arthus reaction is due to accumulation of edema fluid and polymorphonuclear cells in the reaction site. In contrast to this, the delayed reaction is characterized by numerous mononuclear cells at the skin site. While trying to describe the distinguishing features of Arthus and delayed reactions, Davis *et al.* (1967) stated that,

> "The situation is, however, often more complex, and a severe Arthus reaction, which can be hemorrhagic and necrotic, can remain conspicuous for 24 hours or more. The distinction from the delayed-type reaction is then hardly possible by either gross or microscopic inspection."

Since in their experiment Cooper *et al.* (1966a) noted positive wattle reaction at 5 hours, it may be that their chickens were showing Arthus reaction at that time. In the present study, it is also possible that some or all the reactions at 48 hours were in part or wholly Arthus reactions. Some evidence in support of this hypothesis was gathered from chickens infected with *M. bovis* after this question arose. The tuberculin test results were observed at 2, 5, 27 and 48 hours. Almost all the infected chickens that had positive wattle reactions at 48 hours also had responses at 5 hours, generally equal to or larger than the reaction at 48 hours.

Another possible explanation for the tuberculin response in Tx and Tx-X chickens is that many of the Tx chickens had some thymic tissue in the proximity of their thyroid glands. It is possible that some of the cells from the thymic remnants may have seeded into the peripheral system and following infection were specifically sensitized. These cells could then be responsible for the positive tuberculin response. According to the current concept, only a few specifically sensitized cells can initiate, or are required for, a delayed response. Many of the cells that accumulate at the reaction site are nonspecifically affected.

Many of the thymectomized chickens in which no remnant of the thymus could be found also had variable degrees of response to tuberculin. It is possible, but not probable, that some undetected thymic tissue was present. It may also be that some thymus dependent cells in the peripheral system survived X-irradiation. Even distribution of irradiation for all chickens is difficult, but the present method was vastly superior to that used by others. Some variability in the dose received by individual chickens undoubtedly occurs, although it is believed to be negligible. In the present series of experiments considerable care was taken to insure even irradiation. Some indirect evidence in this respect has been presented by Druet and Janigan (1966). These authors reported that bursectomy coupled with X-irradiation did not completely prevent the appearance of pyroninophilic cells in the spleen even though the chickens were adequately bursectomized.

The results of the present study are not in complete agreement with those of Jankovic and Isvaneski (1963). These authors showed that when Tx chickens, sensitized with homologous spinal cord in complete adjuvant, were skin-tested with the spinal cord lipid, only 5 of 7 chickens had positive wattle reaction at 48 hours. The intensity of response in the Tx chickens was markedly reduced in contrast to the severe reaction in control chickens. When Tx chickens were skin-tested with tuberculin, however, 6 of 7 chickens had various degrees of tuberculin response. These Tx chickens reacted to tuberculin more intensely than to spinal cord lipid. The conclusion that can be drawn from this report is that the delayed response in chickens was not completely inhibited, but reduced in intensity following thymus ablation. It should be emphasized that the discrepancy between the reports of Jankovic and Isvaneski (1963) and Cooper and co-workers (1966a) regarding the tuberculin response in Tx chickens would be attributed by some to omission of X-irradiation by Jankovic and Isvaneski.

In the present study, the Tx and Tx-X chickens had approximately equal reactions to tuberculin. Evidently, irradiation following thymectomy did not have any significant effect upon the tuberculin response. Some evidence in this respect has been advanced by Fauser (1969), who showed that the percentage of the vascular lymphocytes was significantly reduced in Tx chickens irrespective of whether they were exposed to irradiation following thymectomy.

In the study using *M. bovis* as the infecting agent, none of the Tx-X chickens had a reaction to tuberculin. Approximately 17% of the Tx infected chickens had tuberculin reaction at 48 hours. Although these data may suggest that the Tx and Tx-X chickens had little or no response to tuberculin, only 20% of control infected and 33% of X-irradiated infected chickens, respectively, had tuberculin response. The tuberculin reaction could have resulted from sensitization due to local multiplication of the organisms in skin lesions which later disappeared.

Thymectomy. In the present study, most of the Tx and Tx-X chickens were found to have thymic tissue in the vicinity of the thyroid gland. It is the belief of this author that complete thymectomy in sufficient numbers of chickens is virtually impossible by the present surgical technique. Thymic tissues were not only in direct contact with the thyroid gland but frequently extended beyond the gland for variable distances. The continuation of thymic tissue with the thyroid gland has been previously reported (Payne, 1952).

Difficulty in achieving complete thymectomy has been experienced by other workers (Warner and Szenberg, 1962; Aspinall *et al.*, 1963; Jankovic and Isvaneski, 1963; Jankovic and Isakovic, 1964; Peterson *et al.*, 1964; Cooper *et al.*, 1966a; Druet and Janigan, 1966). Microscopically, the presence of thymic tissue in the vicinity of the thyroid gland of Tx chickens has been reported. These chickens appeared during gross examination to be otherwise adequately thymectomized (Jankovic and Isakovic, 1964). These authors considered thymectomy satisfactory if the thymic remnants weighed less than 0.1 g.

In their thymectomized chickens, Warner and Szenberg (1962) noted grossly that somewhere between 5 and 10% of thymic tissue was left. Peterson *et al.* (1964) and Cooper *et al.* (1966a) found thymic residua in approximately 4% and 5% of their thymectomized birds, respectively. However, this observation was based only on gross observations. Careful microscopic examination would have undoubtedly revealed a larger proportion of incompletely thymectomized birds. It may also be emphasized that some of the discrepancies between the present investigation and the previous reports may have been due to the different strains of chickens used by different workers. Whether chickens from different strains have different thyroid-thymus relationship is yet to be resolved.

Many of the chickens in the present series of experiments were not completely thymectomized. The amount of thymic substance left in these chickens was very little and was estimated at less than 15%, but probably varied from 5 to 15%. In this study chickens were considered completely thymectomized only when thymic tissue was not detected grossly and microscopically. The question that may be raised is whether chickens showing thymic remnants, even in small amounts, can be called "thymectomized." Certainly this has been done by other workers. It is very difficult to furnish an answer since the manner in which the thymus influences the peripheral system is not known. It is of interest to note that the thymic remnant, no matter how small it is, does not undergo functional alteration even after several months following thymectomy. Thymic remnants do not seem to undergo hypertrophy or hyperplasia as do most other tissues of the body in response to demand for increased function.

It is the belief of this author that most of the Tx chickens in the present series of experiments, irrespective of whether they had thymic remnants, were sufficiently thymectomized functionally although not anatomically. Two evidences essentially justify this statement. First, most of the Tx chickens showed variable degrees of lymphocytic depletion from their spleens. Also, there was a significant decrease in the percentage of vascular lymphocytes in Tx and Tx-X chickens. Furthermore, there was no significant difference in the percentage of vascular lymphocytes between the completely and incompletely thymectomized chickens (Fauser, 1969).

Second, there is precedent in the experiment conducted by Jankovic and Isvaneski (1963) that functional thymectomy may occur in the absence of complete anatomic thymectomy. Two of 7 Tx chickens appeared to be completely thymectomized, yet clinical signs of experimental allergic encephalomyelitis (EAE) were absent from all chickens except in one which was ataxic. In contrast, ataxia and paralysis were noted in most of the Bx and control chickens.

Skin at the site of inoculation. Lesions at the sites of inoculation were seen in all *M. avium*-infected, some Group III-infected but no *M. bovis*-

infected chickens. There was some correlation between the frequency of skin lesions and isolation of acid-fast organisms from the birds. In *M. avium*-infected chickens, acid-fast organisms were isolated from all birds. In Group III-infected chickens, bacterial isolation was accomplished in only 20% of the birds. Acid-fast organisms were not isolated from any *M. bovis*-infected chickens.

There was a correlation between the skin lesions and virulence of the organisms. *Mycobacterium avium* is highly virulent in chickens and it caused skin lesions in all chickens in the present study. *Mycobacterium bovis* is avirulent in chickens or causes only localized lesions. In the present study, no skin lesions were observed in *M. bovis*-infected chickens. Group III organisms are moderately virulent in this species and in the present study caused skin lesions in part (57%) of the birds.

In the *M. avium*-infected chickens, a higher percentage of small lesions was present in Tx-X chickens than in Tx, control or X-irradiated chickens. This observation is in agreement with that of Cooper *et al.* (1966a), who reported that the Tx-X chickens did not have severe adjuvant reaction in their feet. It may, however, be emphasized that none of the Tx-X birds in the present investigation had complete absence of skin lesion at the site of inoculation.

According to Jankovic and Isvaneski (1963), injection of complete Freund's adjuvant into the foot-pads of chickens caused granuloma formation. Whether the granulomatous response was less intense in the Tx chickens was not reported. These authors did not use Tx-X chickens in their experiment.

Lymphocytic cell distribution. In the present study, depletion of small lymphocytes from the spleens of Tx and Tx-X chickens was noted. This is in agreement with the observations of several other investigators (Jankovic and Isakovic, 1964; Cooper *et al.*, 1965; Cooper *et al.*, 1966a). However, there was no direct correlation between the completeness of thymectomy and the amount of lymphocytic depletion as claimed by Jankovic and Isakovic (1964). A few chickens in the present investigation did not have significant lymphocytic depletion from the spleen, although by gross and microscopic examination they appeared to be completely thymectomized. Part of the discrepancy between the present study and the reports of other workers may have been due to the fact that in previous studies all the chickens were examined histologically around 2 months of age. In this study, however, the chickens were examined around 5 months of age, thus giving more time for possible regeneration of the thymus dependent cells. It is also possible that some of the completely Tx chickens, that did not show significant lymphocytic depletion, may have had thymic remnants that were not detected in spite of careful gross and microscopic examinations.

This study is not in agreement with that of Cooper *et al.* (1966a), who noted lymphocytic depletion from the ileocecal junction.

Six *M. avium*-infected chickens had marked depletion of lymphocytes from their spleens. These included 5 X-irradiated chickens and 1 control chicken. The depletion in these cases was diffuse in nature and was more prominent around the Schweigger-Seidel sheaths. This change was not seen in any of the uninfected, X-irradiated chickens. It is, therefore, believed that the depletion noted in these chickens was related to infection and not to X-irradiation. Careful examination revealed that this change was due to periarterial amyloidosis. Changes of this nature were also evident in 2 Tx-X and 1 Tx infected chickens. In these birds, whether depletion was due to the effect of thymectomy or due to diffuse amyloidosis was difficult to resolve. Depletion of lymphocytes from the white pulp of the spleen of chickens injected with azo-casein has been reported (Druet and

Janigan, 1966). This was attributed to amyloid deposition surrounding the Schweigger-Seidel sheaths.

Gross and microscopic lesions. Since most of the Tx and Tx-X chickens in the present series of experiments had positive tuberculin responses, it is difficult to state if they were free of delayed sensitivity. However, since it has been claimed that the thymus is responsible for mediation of delayed hypersensitivity, and neonatal thymectomy coupled with X-irradiation eliminated the delayed response, it is presumed that the Tx and Tx-X chickens in this study were either free from delayed hypersensitivity or at least had an impaired delayed response.

Grossly, there was no significant difference in the numbers of tubercles produced in *M. avium*-infected chickens, irrespective of whether the thymus was removed. This is in accordance with the contention of Rich (1951) that delayed hypersensitivity is not responsible for tubercle formation. However, gross evidence of hepatomegaly and splenomegaly was more prominent in the control, X-irradiated and Tx chickens. Since a relatively small number of Tx chickens was used in this experiment, no definite conclusion can be drawn as to whether the incidence of hepatomegaly and splenomegaly was higher in this group. The hepatic and splenic lesions in some control and X-irradiated chickens also appeared larger in size.

Microscopically, there was a smaller percentage of caseous lesions in Tx and Tx-X chickens than in control and X-irradiated birds. Statistical evaluation revealed that the reduction in the percentage of caseous lesions was due to the effect of thymectomy, and X-irradiation had no significant contribution. There were greater numbers of caseous lesions in the spleen than in the liver, and necrosis was usually more advanced in splenic lesions. This may be because the lesions in the spleen started earlier than in the liver.

In some birds in which gross lesions were present, no microscopic lesions were observed. This was no doubt due to the fact that the lesions had been missed in sectioning for microscopic examination.

It may be concluded from this experiment that thymectomy significantly reduced the amount of tissue necrosis. Whether this effect was due to the absence or impairment of delayed sensitivity cannot be answered at this time. Presuming that this was true, this study provides indirect experimental evidence to support the contention of Rich (1951) and other workers that delayed hypersensitivity is at least partially responsible for tissue necrosis in tuberculosis.

The findings recorded herein are in accordance with the observations of Jankovic and Isvaneski (1963) and Blaw *et al.* (1967), who in separate studies showed that the incidence of EAE was reduced although not completely eliminated in Tx and Tx-X chickens. From the report of Blaw and associates it was surprising to note that of 9 Tx-X chickens used in their study, 4 had clinical signs of the disease. Five of the 9 Tx-x chickens had microscopic evidence of EAE. Theoretically, these chickens should not have shown any evidence of the disease. The reason for this discrepancy was not given. Irradiation coupled with thymectomy did not seem to have any significant effect on the incidence of EAE as compared to those chickens that had undergone thymectomy alone. This raises the question as to whether X-irradiation is really necessary after neonatal thymectomy.

In mammals the thymus is responsible, at least in part, for the mediation of both humoral immunity and delayed hypersensitivity. Accordingly, results of experiments from thymectomized mammals cannot be compared with the results from chickens with thymic ablation. This is evident from the following example. Takeya *et al.* (1967) infected control and Tx mice with *M. tuberculosis*. Mice that had undergone thymectomy had caseous pneumonia, whereas control mice had tubercles consisting of epithelioid cells.

In the present study, amyloid deposition was noted in the liver and spleen of *M. avium*-infected chickens belonging to all 4 groups. Two different patterns of deposition were noted in the spleen. Diffuse amyloidosis about the Schweigger-Seidel sheaths of spleen was seen more often in X-irradiated chickens than in chickens from other groups.

Group III mycobacteria are capable of producing mild systemic disease in chickens. In this experiment only a small number of chickens had local lesions at the site of inoculation. Bacteriologic findings indicated that the organisms multiplied only in a few chickens. It may be concluded that this particular Group III organism is relatively avirulent for chickens, and no increase in the virulence of the organism or susceptibility of the chickens due to thymectomy was detected.

Chickens are not susceptible to infection with *M. bovis*. Experimental inoculations do cause tubercles to develop, but the infection remains limited (Feldman, 1938). In the present study, no local lesions were detected in infected chickens, nor were mycobacteria isolated by standard cultural methods.

SUMMARY AND CONCLUSIONS

The lesions of tuberculosis and the tuberculin reactions were studied in surgically thymectomized and thymectomized X-irradiated chickens infected at 10 weeks of age with mycobacteria.

In *Mycobacterium avium*-infected chickens the lesions at the sites of inoculation were somewhat smaller in the thymectomized X-irradiated chickens.

Tuberculin reactions were evident in almost all infected chickens 48 hours after injection of the tuberculin. Although individual chickens reacted with varying intensity, there was no detectable difference in tuberculin reactions among different groups. However, it is probable that the reactions were due to or complicated by an Arthus type sensitivity.

In thymectomized and thymectomized X-irradiated chickens there were varying degrees of lymphocytic depletion from the spleen.

All groups of chickens infected with a virulent strain of *M. avium* had granulomas in the liver, spleen, ileocecal junction, thymus and lungs. Although there was no essential difference in the cellular makeup of these lesions, caseation necrosis appeared to be less prominent in the thymectomized and thymectomized X-irradiated chickens. There were significantly fewer caseous lesions in the hepatic sections of thymectomized and thymectomized X-irradiated chickens. There were more caseous lesions in the spleen than in the liver. Lesions at the sites of inoculation were seen in all birds infected with *M. avium*.

Among chickens infected with Group III mycobacteria, only small numbers had local lesions at the sites of inoculation, and there were no lesions of tuberculosis in the viscera. Thymectomy and/or X-irradiation did not detectably alter the susceptibility to Group III organisms.

In chickens infected with *Mycobacterium bovis* there were no lesions at the sites of inoculation or in the viscera.

With the present surgical technique, complete thymectomy in all birds was virtually impossible to accomplish. Sometimes the thymic tissue bordered the thyroid grossly and often extended posterior to this gland. Microscopically, thymic lymphocytes were seen adjacent to the thyroid follicles with no sharp line of demarcation between the two.

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