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EFFECTS OF DEVELOPMENTAL HORMONES ON AVIAN

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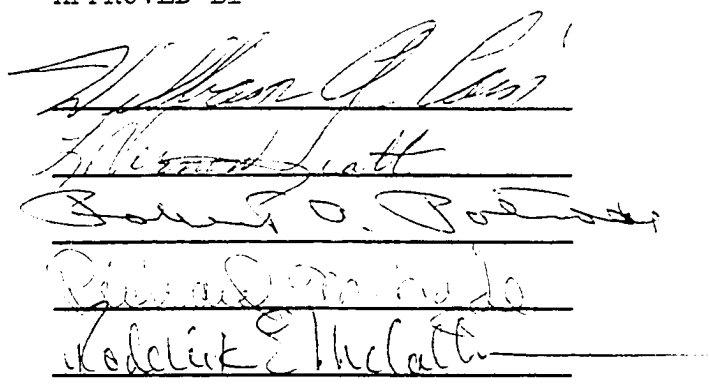

DISSERTATION COMMITTEE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES--Continued

LIST OF TABLES--Continued

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS--Continued

EFFECTS OF DEVELOPMENTAL HORMONES ON AVIAN LYMPHOID TISSUE

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Early anatomists, trying to characterize lymphoid structures such as the thymus in mammals, and the bursa of Fabricius in birds, studied their possible role as endocrine glands. These studies led to a great volume of information about the effects of endocrine glands on the size and morphology of the lymphoid organs. Much knowledge about lymphoid tissue structure and function has now accumulated. With this new knowledge of the function of the lymphoid tissue, the role of the endocrine glands in influencing that function is now being studied.

The purpose of this investigation was to study the interrelationships of the developmental hormones, thyroxine and somatotropic hormone, with the lymphoid tissues of the chicken. These interrelationships were investigated with reference to the ontogeny of the immune response and support of immune function in young and adult animals.

Effects of Sex Steroids and Adrenal Steroids on Lymphatic Tissue

Historically, the effects of hormones on lymphoid tissue have been measured through studies of involution of lymphoid organs. Hammar (74) classed lymphoid involution as accidental or age related. Age involution is that physiological process which begins shortly after puberty as the thymus begins to decrease in size, weight, and lymphoid cellularity. Accidental involution may occur as a result of severe illness, malnutrition or a variety of other stressful situations. Dougherty (34) used the term acute involution to denote the abrupt response of lymphatic tissue to either physiological or pathological stimulation. Although no individual hormone may be implicated in production of age involution, experimental evidence has demonstrated effects of several hormones in causing acute involution of lymphoid tissue.

Gonadal hormones have been shown to alter the state of lymphoid tissue. Testosterone, methyl testosterone, and dehydro-iso-androsterone all cause atrophy of the thymus in rats (139). Testicular extracts injected into castrated rats also cause thymus involution (90). Plagge (126) found testosterone propionate to be four times as effective as testosterone in producing thymus atrophy. Selye (cf. 139) concluded that all hormonally active steroids will produce thymic involution when administered for a sufficient period of time. These compounds cause an acute type involution

rather than increasing the rate of natural involution (126).

Estrogenic compounds have also been studied. Estrogens cause thymic atrophy in rats (67), however, Money et al. (104) found testosterone to be more effective in causing thymic involution than progesterone, estrone, estriol, or diethylstilbestrol. Effects were not as pronounced on lymph nodes as they were on the thymus. Equine gonadotrophic hormone (52) and large doses of progesterone (142) cause thymic atrophy. The effects of oestrone, oestradiol and testosterone were found to be independent of the adrenal gland since they caused involution of the thymus, spleen, and lymph nodes when injected into adrenalectomized animals (136).

The adrenal gland also has an influence on lymphoid tissue. Lymphoid tissue will undergo involution in response to adrenocorticotropic hormone (ACTH) in many species of animals. The rat (148), mouse (35), dog (130), rabbit and human (36) all show a characteristic response to ACTH. Injections of ACTH will not, however, cause lymphoid tissue involution in adrenalectomized animals. Various adrenocortical steroids potentiate the involution of lymphoid tissue when injected into intact or adrenalectomized animals. Money et al. (104) showed that cortisone, 11-dehydrocorticosterone, desoxycorticosterone, and dihydrocortisone all cause thymic involution in the rat. All experiments showed a high correlation between lymphatic tissue weights and the quantity of steroid hormone administered. Cortisone and

11-dehydrocorticosterone were the most effective in reducing the weights of both thymus and lymph nodes. Large doses of desoxycorticosterone acetate will induce thymus atrophy in adrenalectomized rats as well as in intact animals (33).

Because of lymphoid tissue involution in response to adrenal cortical steroids, any agent which caused increased adrenal cortical steroid release could be indirectly responsible for reduction of lymphoid tissue mass. Selye (141) lists operative trauma, burns, hemorrhage, exposure to excessive heat and cold, low atmospheric pressure, and others as agents which increase adrenal cortical secretion. Other factors such as pregnancy and lactation have been found to contribute to thymus involution (70). X-irradiation and radiomimetic drugs, e.g., nitrogen mustards, cause lymphoid tissue atrophy and adrenal cortical stimulation. Thus, at least two mechanisms mediate lymphoid tissue atrophy in animals exposed to irradiation or nitrogen mustard (87, 116). In such animals, adrenalectomy blocks only one of these mechanisms.

As previously discussed, the effects of hormones on lymphatic tissue have been studied by injection of the hormones into intact animals with observation of lymphoid tissue involution. Additional methods of study have involved extirpation of one or more endocrine glands followed by observations of the effect on growth of lymphoid organs. Thymus enlargement occurs after gonadectomy of either sex in

almost every species of domestic and experimental animal and man (cf. 34). Gregoire (cf. 34) found that the rate of return of lymphocytes to normal levels after irradiation was greater in gonadectomized than in intact animals. The effect of adrenalectomy is similar to that of gonadectomy in that both procedures result in increased lymphoid tissue weight over that found in intact animals (98). Gonadectomy and adrenalectomy in the same animal produced greater increases in lymphoid tissue than did either procedure along (98). Data from several authors (cf. 34) indicate that secretions from the adrenal and the gonads exert a similar moderating influence on growth of lymphoid tissue, with the adrenal cortical secretions having the greater influence.

Another method of study of the effect of hormones on lymphoid tissue involves the changes in peripheral blood total leucocyte and lymphocyte counts following alteration in normal hormone levels. In general, these changes are similar to those described for the thymus, spleen, and lymph nodes. Adrenalectomy induces a general lymphocytosis (30, 162). Injection of ACTH, on the other hand, causes lymphopenia in mice (86) and in rats and rabbits (162, 168). In other instances, injections of ACTH or chronic stress may lead to lymphocytosis (30). This may be due to a pituitary reflex which stops ACTH synthesis thus limiting the amount of steroid produced in the adrenal cortex (162). Cortisone also causes a prompt and prolonged fall in the level of

circulating lymphocytes (128). The effect of physiological stress conditions, pregnancy, and lactation on lymphoid tissue is also expressed as lymphopenia in the peripheral blood (42, 75, 124). Some agents, such as X-irradiation (116), and nitrogen mustards (87), may produce lymphopenia directly without mediation by the adrenal glands. Although injection of sex hormones or gonadectomy have profound effects on lymphoid organ weights, little evidence is available about effects on peripheral blood leucocyte numbers (cf. 34).

In summary, sex hormones and adrenal cortical hormones have an antagonistic influence on the growth and cellularity of lymphoid organs. The next section will deal with the endocrine potentiators of lymphoid tissue structure and function.

Effects of Developmental Hormones on Lymphoid Tissue

Thyroxine

Thyroidectomy causes atrophy of lymphoid tissue (98, 131), and exogenous thyroxine administration causes lymphoid hyperplasia (48). Since the effect of thyroxine occurs not only in intact animals but also in adrenalectomized, gonadectomized, adrenalgonadectomized, and hypophysectomized animals, the effect is not mediated by other endocrine glands (cf. 47, 96, 97, 137). Experimental evidence of thymic hyperplasia induced by thyroid hormone is found in

young, well-nourished animals treated with moderate doses of thyroid hormone (58, 131, 146). Massive doses of thyroxine produce thymic atrophy (3, 4).

Other evidence of the hyperplastic effect of thyroid hormone comes from experiments involving rates of reconstitution of involuted lymphoid organs. Gregoire (cf. 34) determined the rate of return to normal weight of lymphoid tissue of rats after they were exposed to whole body X-irradiation. The rate of reconstitution was greater in adrenalectomized rats than in intact X-irradiated rats. The recovery rate was even greater in adrenalectomized and gonadectomized rats. The greatest rate of reconstitution of lymphoid tissue was in adrenalectomized and gonadectomized animals given thyrotropic hormone. Steroid involution of lymphoid tissue was followed by increased rate of regeneration when animals were treated with thyrotropic hormone (6). Gyllensten (73) found thyroxine to stimulate regeneration in the lymph node tissue of young guinea pigs following lymphoid tissue involution caused by administration of a corticosteroid. He indicated that thyroxine stimulates differentiation and proliferation in the tissue. In involuted tissue, thyroxine treatment stimulated growth or proliferation more readily than maturation. In noninvoluted tissue, similar treatment stimulated maturation more readily than it stimulated a growth response.

The cellular changes in thyroxine-induced hyperplasia have been studied in the thymus, lymph nodes, and spleen of

guinea pigs (43, 44, 50, 51). Administration of a single dose of thyroxine produced histological changes in the thymic cortex indicating a proliferation of pyroninophilic cells (43, 50). At this time, an increased output of small lymphocytes from the thymus was seen (51). A slight increase in medullary lymphocytes was also seen (43). The mitotic time for cells in the thymic cortex was decreased along with the increased venous output of small lymphocytes from the thymus (43, 51). This increased output of cells from the thymus probably explains why the increase in weight of the thymus is not as great as the weight increase of lymph nodes and spleen in thyroxine-treated animals (44, 45, 46, 48). A sensitive balance exists between the proliferative capacity of the thymus and its output of lymphocytes (43, 51).

In lymph nodes, the first thyroxine-induced change was an increased number of plasma cells occurring within 24 hours after thyroxine injection (43). At 3, 6, 9, and 16 days after repeated thyroxine injections, a greatly increased number of pyroninophilic cells was correlated with the gain in weight of the lymph nodes (48). This proliferation of cells was also demonstrated by Lundin (96) who reported an increased incorporation of ^{32}P into DNA in thyroxine-stimulated lymphoid tissue.

Thyroxine injections also caused increased numbers of large pyroninophilic cells in the red pulp of the spleen. These changes occurred later than changes in the thymus and

lymph nodes (43, 44, 48). The low mitotic incidence of these cells indicated that they immigrated from the white pulp, blood, or thymus (50). The cells probably did not come directly from the thymus since they appeared with the same regularity in thyroxine-treated thymectomized animals (44).

After establishing the cellular changes induced by thyroxine treatment, Ernstrom (45, 46) performed experiments using thyroid-induced hyperplasia of lymphoid organs to study the role of the thymus in supporting the lymphoid structure and function of peripheral organs. Young guinea pigs were thymectomized and treated with thyroxine either three or 30 days after thymectomy. In thymectomized and sham-thymectomized guinea pigs no significant differences in weight were noted in spleen or lymph nodes. Thyroxine treatment beginning three days after sham-thymectomy resulted in a relative increase in weight of mesenteric lymph nodes, total lymph node mass, and to a lesser degree, spleen. Thyroxine treatment beginning three days after thymectomy also caused increased weights of lymphoid organs. If, however, thyroxine injections were begun 30 days after thymectomy, the thyroxine-induced hyperplasia of lymphoid organs was not seen. In fact, the weights of cervical and extremital lymph nodes were less than in non-treated normal animals. The total lymph node mass of sham-thymectomized guinea pigs treated with thyroxine was 13% greater than that of thymectomized guinea pigs treated with thyroxine. In these

experiments, the difference between the reaction to thyroxine of thymectomized animals and sham-operated animals increased with the time interval between the operation and institution of thyroxine treatment. Removal of the thymus had similar influences on the rate of thyroxine-stimulated regeneration of lymphoid tissue after steroid-induced involution (49).

From these experiments, thyroxine-induced hyperplasia of lymphoid tissue seems to be dependent on the presence of a short lived thymic factor. This could be either a humoral factor or a supply of thymus-dependent cells that makes up the population of the peripheral lymphoid tissue.

Several general conclusions concerning the effects of thyroxine on lymphoid tissues can be listed. Increased numbers of plasma cells in the lymph nodes, increased output of small thymic lymphocytes, and shorter mitotic times indicate increased rates of differentiation of these particular cell lines. These effects are similar to thyroxine-induced metamorphosis, regeneration, and differentiation of different organs in amphibians (109). The increase in numbers of pyroninophilic cells with signs of increased protein synthesis agrees with the effect of thyroxine on protein synthesis in general (101). In addition, changes in lymphoid tissue mass may be mediated directly by thyroxine or by changes in steroid metabolism also induced by thyroxine. Thyroxine causes increased degradation rates of corticosteroids from plasma, probably due to stimulation of hepatic enzyme

activity (13, 14, 55). The reduction of corticosteroids causes an increased release of pituitary ACTH through a feed back mechanism and stimulates the production and release of glucocorticoids from the adrenal gland.

Additional evidence for a thymus-thyroid relationship has been reported (71, 72). Two weeks after thymectomy, an increase in thyroid weight, acinar cell height, and oxygen consumption was observed. Four weeks after thymectomy, thyroid weight, acinar cell height, and oxygen consumption had returned to near normal values (71). This is similar to the observation of increased thyroid weight with the beginning of regeneration of involuted lymphoid tissue (72).

These observations, expecially those of Ernstrom and coworkers (43-51), have provided the ground work for understanding changes in the morphology of lymphoid tissues related to thyroxine activity. The influence of hormones on the function of lymphoid tissue has also been studied.

Nilzen has studied the influence of the guinea pig thyroid on production of precipitating antibody, Schultz-Dale reactivity, respiratory anaphylactic activity (111), allergic encephalitis (110), skin homograft rejection (113), agglutinating antibody (112), and skin reactivity to 2,4-dinitrochlorobenzene (DNCB) (114). Intact or thyroidectomized guinea pigs were injected with egg albumen. Compared to controls, thyroidectomized animals produced no precipitating antibody as detected by a precipitin ring test.

Skin tests of normal animals revealed a reaction after 24 hours characterized by infiltration with lymphocytes and necrosis which was termed Arthus reactivity by the authors. This reaction was not present in thyroidectomized animals. A section of intestine was removed from thyroidectomized and normal animals and challenged with antigen *in vitro*. The Schultz-Dale reaction was weaker in the thyroid-ectomized animals. Respiratory challenge of the animals showed typical anaphylactic reaction in the intact guinea pigs but not in the previously thyroidectomized animals (111). Thyroidectomy in guinea pigs had no effect on the rate or severity of encephalitis produced by injections of homologous brain tissue in adjuvant (110). Thyroidectomized animals rejected skin grafts as rapidly as did intact animals (113). Thyroidectomy did, however, have a slight influence on antibody production against *Salmonella typhi* (112). The rise of antibody levels in the serum of thyroidectomized animals occurred at a slower rate, although the maximum quantity of antibody attained was the same as that observed in intact animals. Only a few points on the antibody response curve of thyroidectomized animals were significantly different from the response of intact animals (112). Some relationships of the thyroid to skin sensitization with DNCB were also studied (114). Large doses of thyroxine caused decreased reactivity to skin sensitization. This was thought to be due to increased hyperfunction of the adrenals in these animals since

the adrenal glands had increased cellularity. Smaller doses of thyroxine caused increased sensitivity to DNCB in treated animals compared with untreated controls. This increase was expressed as a shorter lag time in the development of sensitivity as well as more severe skin reactions. Thyroidectomy, however, did not lower DNCB reactivity when compared to intact animals (114). Yuri (169) found thyroidectomy to decrease inflammatory symptoms and circulating antibody titers in experimentally-induced allergic conjuctivitis in rabbits. Thyroidectomy decreased and thyroxine restored the polyarthritic response induced by Mycobacterium butyricum adjuvant in rats (150). Long and Miles (94) studied endocrine influences in establishing "bacterial allergy," now known as delayed hypersensitivity. B. C. G. infected guinea pigs were treated with thyroxine, cortisone, or ACTH and challenged with a tuberculin skin test. Thyroxine increased the skin reaction while cortisone and ACTH treatment lessened the reaction. Two other important observations were made. If thyroxine treatment was stopped for two weeks, the animals became less hypersensitive than animals that were never treated with thyroxine. On the other hand, animals became more hypersensitive two weeks after cortisone or ACTH injections had been stopped. This was considered to be a compensatory effect of the thyroid and adrenal glands.

Long and Shewell (95) found immunity to diphtheria toxin to be greater in guinea pigs previously injected with

diphtheria toxoid and thyroxine as compared with that in animals injected with toxoid alone. Thyroxine injections also increased the amount of antitoxin produced in guinea pigs (145). Lower antitoxin levels in thyroidectomized guinea pigs were noted as compared with antitoxin levels observed in normal animals. A species difference seems to exist with respect to the influence of the thyroid on antitoxin production against diphtheria toxoid (145). In contrast to the thyroxine-induced increase in antitoxin in guinea pigs; rats, rabbits, and mice produce lower than normal amounts of antitoxin when treated with thyroxine. Thyroidectomy, on the other hand, causes increased antitoxin levels in these animals. The species grouping of this response compares with the response of these animals to cortisone (144). Rabbits, mice, and rats are cortisone sensitive, and lose body weight and capacity to form antitoxin when injected with cortisone. Guinea pigs, humans, and monkeys can withstand the same doses of cortisone without losing body weight or antitoxin producing capacity.

The species differences in circulating antitoxin levels after injection of thyroxine can be explained by studies on the catabolism of gamma globulin in these species (56, 57). The rate of catabolism of homologous gamma globulin injected into rats or guinea pigs can be altered by changes in the thyroid activity. In rats, the biological half-life of homologous ^{131}I gamma globulin was 4.8 days in

euthyroid, 5.8 days in thyroidectomized animals, and 3.8 days in thyroxine-treated animals (56). Total serum protein levels were not affected by thyroxine injections but were increased after thyroidectomy. This increase was mainly due to increased gamma globulin levels. Catabolism of gamma globulin in thyroidectomized rats is decreased to a greater extent than is gamma globulin synthesis and could lead to increased levels of antitoxin. In guinea pigs, thyroidectomy does not decrease the catabolism of injected gamma globulin (57). Thyroixine injections increased catabolism, however, a larger increase in gamma globulin production was noted.

The response of the immune system to thyroidectomy or exogenous thyroxine administration is dependent not only on the direct effect of thyroxine on lymphoid tissues but also on changes in other endocrine glands and changes in metabolism that could alter the normal degradation rates of gamma globulins. The alteration of the immune response by thyroxine is dependent on many endocrinological changes. Similarly, the effects of other hormones on the immune system may be direct or indirect, and may or may not involve changes in thyroid activity.

Somatotropic Hormone

Experimental approaches similar to those used to study the role of the thyroid in altering lymphoid tissue morphology have been applied to study the influence of the hypophysis on lymphoid tissue. Eartly and Leblond (41)

reported a significant absolute and a slight relative weight (organ weight/body weight) decrease in the rat thymus after hypophysectomy. Feldman (58) showed that the absolute weight of the thymus dropped after hypophysectomy although the weight was slightly increased in proportion to the body weight. The rate of thymus regeneration after involution with ACTH was greater in intact rats than in hypophysectomized animals (19, 20). The recovery of total leucocyte count, hemagglutinating antibody formation to sheep erythrocytes and skin allograft rejection was defective after sublethal irradiation in adult hypophysectomized rats (40). A marked reduction in DNA synthesis also occurs in the thymus of rats after hypophysectomy (115).

Lymph nodes show little change in weight after hypophysectomy. Nagreda and Kaplan (107) found unchanged absolute weights of lymph nodes after hypophysectomy. Feldman (58) noted increases in both absolute and relative weights of lymph nodes of hypophysectomized rats. In contrast to the increased lymph node weights in these experiments, hypophysectomy resulted in decreased spleen weights (19, 58, 107).

Lymphoid hyperplasia of spleen and thymus which occurs after adrenalectomy was inhibited by hypophysectomy (96). In addition, smaller amounts of cortisone induced atrophy of the spleen and thymus if rats were previously hypophysectomized.

An effect of injections of the hypophyseal hormone,

somatotropic or growth hormone (STH), on the lymphoid tissue of intact animals is more difficult to demonstrate. Some of the STH preparations in earlier studies may have been contaminated with thyrotropic hormone and thus caused increased lymphoid tissue weights through increased thyroid activity. Li and Evans (91) reported the ability of STH to restore lymphoid tissue weights after hypophysectomy in rats. In intact animals, STH will stimulate spleen growth above normal weight if the intake of food in the animals is restricted to the amount taken voluntarily by untreated hypophysectomized animals (69). Lundin (96) also reported increased spleen and thymus weights in animals treated with STH. Lymph node weights were not changed. The histological appearance of lymphoid tissue was the same as in untreated animals with the exception of follicular stimulation in the spleen characterized by broadened perifollicular zones and increased numbers of plasma cells. After hypophysectomy, these areas were decreased in the spleen and distinct cortical atrophy was observed in the thymus.

Since both thyroxine and STH may separately stimulate proliferation of lymphoid tissue, their combined effects have been studied (96, 138). Treatment of rats with combinations of STH and thyroxine resulted in increased body weight, but stimulation of the spleen and lymph nodes was not greater than that produced by thyroxine alone (138). STH did not potentiate the effect of thyroxine on lymphoid tissue (96).

Bois and Selye (18) did, however, report that thyroxine exerted a strong action on the adrenal stimulating effect of STH. Furthermore, increased cortical output by the adrenals might antagonize any combined effect of STH and thyroxine on the lymphoid tissue.

The relationship of the hypophysis with the thymus was further studied through histological observations of the pituitary gland after thymectomy in mice (118, 119). Observation by both light (119) and electron (118) microscopy, revealed that a degranulation of the acidophilic growth hormone-producing cells occurs in the adenohypophysis of mice after neonatal thymectomy. This change occurred after thymectomy of neonatal mice and not after thymectomy of young adult mice or after neonatal splenectomy. This indicates that the influence of the hypophysis on the thymus may be stronger during the perinatal period.

This relationship between the thymus and hypophysis was also studied by injecting anti-hypophysis serum into young adult mice (120). A single intraperitoneal injection of anti-hypophysis serum into young adult mice resulted in thymic atrophy, loss of body weight, and severe wasting disease with death in some animals. The wasting disease appears similar to that observed after neonatal thymectomy (102, 103). Injection of normal rabbit serum or anti-thymus serum had no effect on the mice. Anti-mouse pituitary serum was demonstrated by fluorescent antibody techniques to have

affinity for the acidophilic growth hormone-producing cells of the anterior pituitary (123). It also had anti-growth hormone activity and produced changes in the thyroid glands of young mice.

Because of the demonstrated affinity of anti-pituitary serum for STH, experiments were performed to study the effect of heterologous anti-growth hormone (ASTH) serum on the thymus and peripheral lymphatic tissue in mice (121, 122). Repeated injections of rabbit anti-bovine somatotropic hormone (ASTH) globulins into two- and three-week-old mice produced inhibition of body growth, thymic atrophy, involution of lymphoid tissue in the spleen, and a wasting syndrome. Injection of ASTH into adult mice produced similar lymphoid tissue alterations, but wasting syndrome was not observed. Similar treatment with normal rabbit serum globulins produced a normal, antigen-induced, splenic cellular reaction with no wasting syndrome. Simultaneous injection of STH and anti-STH into mice completely reversed the effects of the anti-STH globulins.

Perhaps the most convincing evidence for the role of the pituitary in support of normal lymphoid tissue structure and function is derived from studies of two strains of dwarf mice. These mice have hypopituitary function, with low levels of somatotropic hormone and thyrotropic hormone, and are immunodeficient by several criteria. Snell-Bagg dwarf mice exhibit pituitary dwarfism (genetic symbol dw) which is

a recessive trait characterized by defective development of the pituitary (7). The thymus of these mice shows normal absolute and relative weight (thymus/body weight) until 15 days of age. After 15 days of age, normal animals increase in absolute thymus weight, while dwarf mice show no gain in thymus weight (53). The thymus of Snell-Bagg dwarf mice contains cells of slower mitotic activity than cells from the thymus of normal animals (7), and there is reduced cellularity, especially in the cortex (8). Early involution of the thymus was observed, and a lowered output of thymic cells was postulated (7).

Spleen and lymph nodes of dwarf mice show signs of underdevelopment as determined by size and histological appearance (53). Size and number of lymphoid follicles is deficient throughout life. The parafollicular, thymus-dependent areas of lymph nodes and the periarteriolar lymphoid sheaths of the spleen are underpopulated. The red pulp of the spleen also shows reduced numbers of mature and immature plasma cells. Peripheral leucocyte levels are lower in dwarf mice than in normal litter mates throughout life (8).

Antibody plaque forming capacity was also found to be deficient in dwarf mice injected with sheep erythrocytes (8). The plaque forming response did not mature with age (9). Even though the antibody response to sheep erythrocytes was below normal, pituitary dwarf mice have normal serum immunoglobulin levels (164). Pituitary dwarf mice have a lowered

capacity to reject foreign skin grafts (118), but they have been shown to possess normal responsiveness to picryl chloride (11).

Since the immunological capacity of pituitary dwarf mice is impaired, studies have been performed to test the ability of STH and thyroxine to restore the morphology of lymphoid tissue and the immune response (10, 54, 117). Somatotropic hormone and thyroxine injections can prevent dwarfism, reconstitute central and peripheral lymphoid tissue morphology, and produce recovery of humoral responsiveness (10) and ability to reject skin grafts (54). This recovery lasts for an extended period of time (117, 54). Treatment of dwarf mice for one month with STH and thyroxine restored their immune system with antibody forming capacity and the ability to reject skin grafts for more than one year. Untreated dwarf mice usually died between 40 and 150 days (118). Normal skin graft rejection times and antibody forming capacity can be restored to dwarf mice through injection of lymph node lymphocytes but not thymocytes or thymocytes and bone marrow cells (54). STH and thyroxine do not restore immune capacity to dwarf mice that have been thymectomized.

Another means of reconstituting the immune capacity of the dwarf mice has been explored by Duquesnoy (37, 39). Prolonged nursing of the dwarf mice on foster mothers prevented the appearance of the immune deficiency symptoms which usually begin about the time of weaning. Since dwarf mice

have no deficiency in enzymes in the intestine, the added nutrition of prolonged nursing probably played no role in the effect on the lymphoid tissue of the dwarf mice. A specific factor in mouse milk may act in some way directly on the lymphoid system. However, there are no reports in the literature to indicate the presence of pituitary hormone in mouse milk (37). Thyroxine levels are increased in pregnant and lactating animals (70) and thyroxine may in some instances cross the placenta (84).

A second strain of dwarf mice has been studied (38). The Ames dwarf mouse (genetic symbol df) has an autosomal recessive trait characterized by hypopituitary function. It is similar to the Snell-Bagg dwarf mouse endocrinologically and immunologically. This strain is deficient with respect to peripheral blood lymphocyte numbers, cellular makeup of the thymus and peripheral lymphoid tissue, antibody plaque-forming capacity with sheep erythrocytes as antigen, and graft versus host reactivity of spleen cells. Normal colony forming capacity of bone marrow cells indicated a normal stem cell population in these dwarf mice. Immunoglobulin levels were elevated for IgG, slightly low for IgM and clearly low for IgA. In general, studies in both strains of dwarf mice indicate a definite control of the pituitary over normal growth and function of the immune system in mice.

The role of developmental hormones in support of lymphoid tissue function has been studied with respect to

the ability of STH and thyroxine to influence differentiation and proliferation of thymus-dependent cells. Pierpaoli, Fabris and Sorkin (118) studied the ability of STH and thyroxine to influence runt disease of newborn mice in a graft versus host assay system. Spleen cells from adult C_3H mice were injected intravenously into newborn Charles River recipients. Some mice were treated with STH and thyroxine while others were untreated. Mice injected with the hormones developed an accelerated runt disease and died within 7 to 15 days of age. Untreated mice injected with spleen cells developed runt disease a few days later, but some recovered. Thus, STH and thyroxine have the ability to increase the severity of runt disease induced by spleen cells.

STH also has the capacity to induce immunocompetence in thymocytes of adult or newborn mice (118). Thymocytes from newborn animals of one parental strain of $(C_3$ H X CR$)F_1$ hybrids were injected into newborn $(C_3H$ X CR$)F_1$ hybrid recipients. Thymocytes from newborn animals normally elicit very little graft versus host activity in these strain combinations. If the recipients were injected with STH, thymocytes behaved as immunocompetent cells and induced a graft versus host reaction. Since these results could be repeated in thymectomized recipients, the action of STH on donor thymocytes is probably not aided or mediated through the host thymus.

In summary, much experimental evidence indicates that

developmental hormones, thyroxine and STH, have an influence on the development and function of the immune system. Their role has been studied using many biological measurements and test systems. Because of the varying sensitivities of these determinations, the role of developmental hormones in support of immune function cannot be determined as absolute or quantitative. Further problems arise when one trys to assign the contribution of thyroxine or STH to each of the observations made.

Difficulty is also encountered when one trys to determine the population of cells (thymus dependent or independent) or the site of action (central or peripheral lymphoid tissue) on which the hormones act directly. Because of these interpretive problems a study system in which the normal differentiation, proliferation and function of different cell populations is well characterized would be useful. The use of the chicken as an experimental animal provides such a model.

Effects of Hormones on the Lymphoid System of the Chicken

The development and function of the avian central lymphoid organs, bursa of Fabricius and thymus, are characterized in reviews by Cooper et al. (27), Warner (158), and Jankovic (79). This discussion will concentrate on description of events in development of these systems which might be susceptible to endocrine regulation of immunological

function. Of prime importance in discussion of central lymphoid organs is the concept of vertical and horizontal division of the lymphoid system (27). Definition of the thymus and bursa as central lymphoid tissue as contrasted to the peripheral lymphoid tissue, spleen and lymph nodes, is the concept of vertical division (68). Horizontal division is defined as a compartmentalization of two distinct lymphoid systems (27). The functions of the thymus-dependent lymphoid system are homograft rejection, graft versus host reactivity, delayed hypersensitivity, and contact sensitivity (28, 29). Germinal centers, plasma cells, immunoglobulin production, and specific antibody formation are dependent on the bursa of Fabricius in chickens (28, 29) and on a bursa equivalent in mammals (27). Removal of the thymus or bursa immediately after hatching or after destruction of peripheral lymphoid elements by various means, results in deficiency of the population of cells dependent on that particular central lymphoid organ (28, 29).

The function of the thymus and bursa as central lymphoid organs may be accomplished through similar mechanisms. The thymus may influence cells derived internally, or from other areas of the body (5). Cells which have received such influence(s) seed to peripheral lymphoid tissue. Central lymphoid organs may also support differentiation of the cells in the peripheral tissue through humoral factor(s) (154, 163).

The lymphoid cells of the bursa are derived from blood borne progenitor cells of mesenchymal origin (105, 106). These cells, after bursal influence, migrate to the peripheral tissues (167). Evidence also exists for a bursal humoral factor which may influence differentiation of bursa-dependent cells in the peripheral tissue (82, 83, 135).

The mechanisms through which the thymus and bursa accomplish their functions as central lymphoid organs provide many stages in development which could be influenced or altered by antagonistic or potentiating influences. The effects of various hormones, steroids as well as developmental hormones, on lymphoid tissues of the chicken have been studied in much the same way as in mammals, although not as extensively.

The bursa and thymus of chickens are subject to age and acute involution in much the same way as are lymphoid organs of mammals. The bursa and thymus undergo involution about the time of sexual maturity in birds (132). The thymus remains as a small atrophic organ, with very little cortex, while the bursa undergoes a more complete involution (77). Glick found the normal growth of the bursa to be related to the growth of the testes (61). He suggested that testicular and adrenal hormones are factors in regression of the bursa. The rate of involution of the bursa can be increased with injections of testosterone propionate (88). Reduction of bursa and thymus weights in response to sex hormones was also reported by Selye (140) and Glick (62).

In addition to the influence of sex hormones on lymphoid tissue in the chicken, adrenal steroids affect lymphoid tissue. Injections of cortisone acetate (62, 64) and cortisone, hydrocortisone and corticosterone into chickens caused bursa involution (170). The effect of these steroids is dose dependent (140, 170). Adrenocorticotropic hormone acts through the adrenal gland in reducing the size of the bursa (78, 147).

The role of thyroxine and somatotropic hormone in regulating lymphoid tissue has not been as well documented as the effects of the previously discussed agents. Bovine somatotropic hormone has been reported to have no influence on body weight of chickens (93). Glick (63), however, reported that bovine growth hormone has a stimulating influence on bursa weight and prevents involution of the bursa in response to cortisone injections. Antagonistic effects of cortisone and growth hormone have also been reported in the developing chick embryo (149).

Thyroxine injections did not cause hyperplasia of the thymus, although aminothiazol-induced hypothyroidism caused atrophy of the thymus (78). Garren and Schaffner (60) reported that thiouracil treatment induced hypothyroidism and atrophy of the spleen and bursa. Thyroxine and growth hormone, alone or in combination, increased bursa weight in hypophysectomized birds (12). Injections of thyroxine have also been used to alter the amount of cortical area in the thymus of chickens (157).

Sex steroid-induced arrest of bursa development in embryos has been one of the most useful procedures in immunobiology. Injection of androgens into the chick embryo before lymphocytogenesis begins prevents any lymphoid development in the bursa (100). If the hormone is injected on the 5th or 6th day of incubation, the entire bursal structure fails to develop, and if the hormone is injected on the 11th to 13th embryonic day, lymphoid development in the epithelial bursa is prevented (159).

This procedure, called hormonal bursectomy, prevents the bursa from carrying out its function, and thus results in deficiency of bursa-dependent cells and function. Most hormonally bursectomized birds have normal thymus development. In some birds, however, atrophy of the thymic cortex and impaired development of the thymus-dependent system occurs (160). Hormonal bursectomy occurs at the central lymphoid tissue level and results in lack of development of bursa-dependent peripheral tissue. This destruction of the bursa, if complete, results in permanent impairment of antibody and immunoglobulin synthesis (161).

Hormonal bursectomy before hatching is distinctly different from involution of the bursa, thymus, and peripheral tissue after hatching. Glick (65, 66) found that injection of cortisone acetate or testosterone propionate into chicks for the first four days after hatching produced bursa regression. Thymus regression occurred with cortisone acetate

but not testosterone propionate. Regeneration of the thymus, bursa, and peripheral tissue was complete two weeks after stopping cortisone acetate treatment and 3 to 5 weeks after testosterone propionate treatment. Thus, involution by sex steroids can be divided into: 1) central tissue destruction which prevents development if accomplished early enough in embryonic life, and 2) central and peripheral tissue involution which may regenerate.

Even though the study of the influence of hormones on lymphoid tissue has not been as extensive in chickens as in mammals, the chicken provides an ideal experimental animal because of its clear vertical and horizontal division of the immune system. The ontogeny of these systems can now be studied with respect to the influence of the developmental hormones, thyroxine and somatotropic hormone.

The purpose of this investigation was to study the interrelationships of the developmental hormones, thyroxine and somatotropic hormone, with the lymphoid tissues of the chicken. These interrelationships were investigated with reference to the ontogeny of the immune response and support on immune function in young and adult animals. More specifically, levels of endocrine secretions were altered by injection of specific hormones or antibodies against endocrine gland tissue, surgical extirpation of an endocrine gland, or pharmacologic inhibition of an endocrine gland. The direct or indirect effects of these procedures on

development or maintenance of specific components of immunological competence were evaluated. Findings from these experiments were compared with findings of other investigators.

CHAPTER II

MATERIALS AND METHODS

Experimental Animals

Chickens

Fertile White Leghorn eggs were obtained from Hyline Poultry Farms, Johnston, Iowa, and from Capitol Hill Hatchery, Oklahoma City, Oklahoma. Choice of sources was dictated by budgetary considerations. The more desirable eggs (those from Hyline Poultry Farms) are designated as Line 96. Line 96 is an F_1 or hybrid line prepared by crossing two relatively inbred lines. Each of the parental lines has the major histocompatability genotype, B^2B^2. While other histocompatability loci are not well defined in this line, there is minimal histoincompatability among individuals within the line (22, 23). Eggs from Capitol Hill Hatchery were not well defined for research purposes. It was assumed that these eggs were heterozygous for histocompatability loci since no selection or monitoring of transplantation characteristics had been used.

All eggs were incubated at 39 C in a rotary incubator (Humidaire Incubator Co.). Chicks were fed Purina Startena

chick feed until 4 weeks of age and were then fed Purina Growena ad libitum. Birds were housed in a brooder until 5 weeks of age and then caged in rabbit cages.

Rabbits

New Zealand White, male rabbits were obtained from a local source and were raised in individual cages until 10 weeks of age. They were fed Purina Rabbit Chow and water ad libitum.

Injectables

Testosterone

Three preparations of testosterone were used. Two-tenths milliliter aqueous testosterone, 10 mg/ml (Towne, Paulsen and Co., Inc.) was injected into the allantoic cavity of eggs on the 13th day of incubation. Testosterone propionate, 100 mg/ml (Wollins Pharmacal Corp.) was diluted to 25 mg/ml in sesame oil (Fisher Scientific Co.) and 0.1 ml was injected into the allantoic cavity of embryonated eggs on the 12th day of incubation. Testosterone propionate (Calbiochem) was suspended in sesame oil (25 mg/ml) and 0.1 ml was injected into the allantoic cavity of embryonated eggs on the 12th day of incubation.

Thyroxine

L-thyroxine (sodium) pentahydrate (Nutritional Biochemical Corporation) was dissolved in pH 11 isotonic saline and injected in 0.1 ml volume into the allantoic cavity of

chick feed until 4 weeks of age and were then fed Purina Growena ad libitum. Birds were housed in a brooder until 5 weeks of age and then caged in rabbit cages.

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Thyroxine

Varying amounts of L-thyroxine (sodium) pentahydrate (Nutritional Biochemical Corporation) were dissolved in pH 11 isotonic saline and injected in 0.1 ml volume into the

allantoic cavity of embryos or intramuscularly into birds after hatching.

Thiouracil

One-tenth ml of 2-thiouracil (4-oxy-2-thiopyrimidine) B grade (Calbiochem) suspended in sesame oil in concentrations of 1 mg/ml to 40 mg/ml was injected into the allantoic cavity of 5- or 12-day-old embryos. Amounts of 1 mg/day to 10 mg/day were injected intramuscularly into 5-week-old birds. Thiouracil was also mixed 0.1% (w/w) in the feed and fed ad libitum from the time of hatching.

Somatotropic Hormone

Raben-type bovine somatotropic hormone (Nutritional Biochemicals Corporation) was suspended in isotonic saline, pH 11, and injected in 1 to 4 mg amounts intramuscularly into chicks ranging in age from 1 day to 5 weeks. The hormone was not completely dissolved at this pH, however daily injections of more alkaline solutions caused necrosis of muscle tissue.

Preparation of Antigens

Sheep Erythrocytes

Sheep erythrocytes suspended in Alsever's solution (Colorado Serum Co. Laboratories) were washed three times in isotonic saline by centrifugation of 3000 rpm (1000 X G) in a model HN-S International centrifuge equipped with a number 215 horizontal head (International Equipment Co.). Cells were resuspended to 10% (v/v) in isotonic saline. All birds

were injected intramuscularly with a 0.5 ml 10% sheep erythrocyte suspension unless otherwise stated.

Salmonella "O" Antigen

Salmonella "O" antigen was prepared according to the procedure outlined by Campbell et al. (24). The final suspension of organisms was adjusted to contain 4×10^{10} cells/ml.

Injection and Bleeding of Animals

Embryonated eggs were injected into the allantoic cavity with a 26 gauge needle. After hatching, birds were injected subcutaneously or intramuscularly in the leg, using a 26 gauge needle. Chicks less than one week of age were bled by cardiac puncture. Older birds were bled from the wing vein with a 23 or 26 gauge needle. All blood was collected through needles rinsed previously in sodium heparin, 1000 units/ml (Organon Inc.). The plasma was separated by centrifugation and stored at -20 C. On thawing, the fibrin clot was removed and the remaining serum was used.

Rabbits were injected intracutaneously, subcutaneously, and intramuscularly with a 22 gauge needle. They were bled through the central ear artery with a 20 gauge needle. The blood was allowed to clot at room temperature and the serum was separated and stored at -20 C.

Preparation of Antisera

Anti-Somatotropic Hormone

Two rabbits were each injected intracutaneously with 10 mg bovine somatotropic hormone (STH) distributed in 15 to 30 sites. Each animal received a mixture made with 3 ml STH in distilled water, pH 11, emulsified with 3 ml complete Freund's adjuvant (Difco Laboratories). The same amount of antigen was given one week later in incomplete Freund's adjuvant. The third and fourth weeks, each rabbit received an intramuscular injection of 1 mg STH in alkaline (pH 11) isotonic saline. Each rabbit was bled weekly from the 4th to the 8th week after the first antigen injection.

Anti-Chicken Pituitary Extract

Eighty-five pituitary glands were collected by the method of Garren (59) from birds ranging in age from 3 to 6 weeks. They were pooled and frozen at -20 C in 6 ml isotonic saline. The pituitaries were then homogenized in a glass tissue homogenizer, and the preparation was centrifuged at 2000 rpm (450 X G) for 5 minutes. The supernatant phase was divided into two fractions after a second centrifugation (2000 rpm for 5 minutes) and these fractions were labelled stock and sediment. The stock (supernatant) solution contained 14.5 mg protein/ml as determined by a 260-280 nanometer reading on a Gilford 2400 spectrophotometer (85). One and three tenths milliliters of stock solution were mixed with the sediment from the second centrifugation and

emulsified in an equal volume of complete Freund's adjuvant and injected intradermally into 15 sites in each of two, three kilogram rabbits. A second injection of 18 mg protein was given subcutaneously to each rabbit 20 days later. Both rabbits were bled by heart puncture 15 days after the second injection of antigen. All sera were pooled and gamma globulin was precipitated by the sodium sulfate method described by Deutsch (32). The final globulin preparation obtained from the immune sera contained 40 mg protein/ml. The final globulin preparation obtained from non-immune pre-bleeding sera of these same rabbits contained 26 mg protein/ml.

Assays of the Immune Response

Humoral Immunity

Direct hemagglutination. Serial, two-fold dilutions of sera were made in microtiter "U" plates (Linbro Chemical Co.) using sodium phosphate buffer, pH 7.0 (165) containing 5% human serum which had been heat inactivated at 56 C for 30 minutes. Serum and diluent volumes were 0.025 ml/well. Sheep erythrocytes were washed three times in physiological saline as described previously, resuspended to 0.5% in microtiter buffer, and 0.025 ml were added to each well. Plates were allowed to incubate at room temperature and the reactions were read after 2 hours. Serum samples were titrated in duplicate and the titers were recorded as the average of the two determinations if the titers did not differ by more than two wells. When larger discrepancies were observed,

samples were retitrated. The titer of a serum sample was designated as the $\log_2$ unit of the highest dilution of serum that would agglutinate the sheep erythrocytes. For comparison, a row of wells containing only cells and buffer was included on each plate.

Indirect hemagglutination. After direct hemagglutination titers were recorded, sheep erythrocytes were resuspended by gentle vibration on a Genie vortex mixer (Scientific Industries Inc.). To each well was added 0.025 ml of a 1:20 dilution of rabbit antiserum to chicken gamma globulins according to the method of Cain et al. (21). The plates were again incubated at room temperature for 2 hours and the indirect titer was recorded. The antiserum against chicken gamma globulins was prepared according to the methods of Benedict (15).

Bacterial agglutination. Bacterial agglutination was performed using the methods described for direct hemagglutination except that a 1:60 dilution of the stock Salmonella "O" antigen was used as the antigen. This contained 3.6×10^8 organisms per milliliter of saline. Bacterial agglutination was performed in saline rather than in microtiter buffer.

Passive cutaneous anaphylaxis. Passive cutaneous anaphylaxis (PCA) was performed by modification of techniques presented by Campbell et al. (26). Two-fold dilutions of anti-somatotropic hormone sera were made in saline and

injected intradermally into individual sites on the shaved back of a guinea pig. Three hours later, 4 mg STH, dissolved in 0.5 ml of alkaline saline and mixed with 0.5 ml of 0.5% Evans blue dye (Warner-Lambert Co.) were injected into the guinea pig via heart puncture. After one hour, the reactions at each skin test site were recorded as 0, +, ++, or +++ according to the diameter of the reaction. The PCA titer of the serum was recorded as the highest dilution of serum that showed a + (0.3 - 0.7 cm) reaction.

PCA inhibition was performed by incubating a serum sample having a PCA titer of 320 with saline, STH (in alkaline saline), alkaline saline, young chicken (3 weeks old) serum, adult chicken (6 months old) serum, chicken pituitary extract, or mouse serum before PCA titration as previously described. One-tenth ml of antiserum was added to 0.9 ml of each of the above materials. After incubation at room temperature for one hour, and then at 4 C for 20 hours, the mixtures were centrifuged at 10,000 rpm in a Sorvall RC2-B centrifuge (Ivan Sorvall Inc.) equipped with a type SS 34 head. The supernatant fluid was then removed and each sample was diluted in saline and injected into skin test sites. The PCA titration was performed as described previously.

Double diffusion precipitation. Double diffusion was performed according to the methods of Campbell et al. (25). Two and five-tenths ml of 1.5% (w/v) Noble special agar in

sodium phosphate buffer, pH 7.0 (165), was poured onto microscope slides and allowed to harden. A pattern containing one central and 6 peripheral wells was used. Serum or antigen dilutions were placed in the peripheral wells with the corresponding antigen or antibody in the central well. Precipitin lines were observed after 24 to 48 hours incubation at room temperature in a moist chamber.

Cellular Immunity

Skin grafting. Skin grafting was accomplished by modification of the techniques used by Polley et al. (127). Six-week-old birds were anesthetized with 0.3 ml Dibutol, 60 mg/ml (Sodium Pentabarbitol, Diamond Laboratories), injected intramuscularly. Feathers were removed from their backs and the skin was cleaned with 70% ethanol. The skin was then coated with flexible collodion (Fisher Scientific Co.) and overlayed with two thicknesses of cotton gauze. The gauze was saturated with more collodion and allowed to dry. One centimeter squares were marked on the gauze. The squares were removed by cutting through the gauze and the first layer of skin. Autografts were turned 180 degrees and replaced. Allografts were usually taken from a single donor. The allografts were from a bird of similar B histocompatibility genotype, in line, or were from an out of line donor. The graft adhered to the graft bed without sutures and it was covered with a plastic bandage (Curad, Kendall Co.). The entire back was then covered with gauze and secured with

adhevise tape. Bandages and the collodion gauze were removed after 6 or 8 days. Grafts were observed daily for signs of rejection and a graft was determined rejected when it was a brownish black color and shrunken, according to the criteria of Polley et al. (127). This type of graft is soon sloughed completely. Most allografts were near this rejection stage at the time of removal of the bandages.

Histological Studies

Organs were fixed in 10% (v/v) neutral buffered formalin and processed in an automatic tissue processor (Auto-Technicon). Sections were cut from paraffin blocks at a thickness of 7 microns and were stained with hematoxylin and eosin.

Hematological Studies

Total peripheral blood leucocyte levels were determined using the methods of Natt and Herrick (108). Leucocytes were classed as heterophils, eosinophils, basophils, and lymphocytes according to descriptions presented by Sturkie (151).

Ultracentrifugation

Fractionation of serum was carried out according to methods of Trautman and Cowan (155). Individual serum samples were fractionated on continuous linear 10% to 40% (weight/volume) sucrose density gradients by centrifugation at 100,000 X G (35,000 rpm) for 18 hours at 10 C in a

Beckman model L2-65B ultracentrifuge with an SW 39L rotor. Serum (0.2 ml) was diluted with an equal volume of 10% sucrose and layered over the gradient. After centrifugation, fractions were collected by puncturing the bottom of the centrifuge tube and allowing the contents to drip out. Each fraction contained seven drops collected serially. Protein concentration profiles were recorded using a 280 millimicron Uvicord absorbancy plotter (LKB Instruments Inc.) and a Sargent recorder, model SRG (E. H. Sargent and Co.).

Statistical Analyses

Statistical analyses were made using a one-tailed Student T test according to Li (92). Differences between means were considered to be significant if $p \leq 0.05$.

Experimental Design

Effect of Thyroxine on Testosterone Treated Embryos

The effect of thyroxine on embryos injected previously with testosterone was studied. Two-tenths ml of a 10 mg/ml solution of aqueous testosterone (Towne, Paulsen and Co., Inc.) was injected into the allantoic cavity of eggs on the 13th day of incubation. A second group of embryos received testosterone on the 13th embryonic day followed by two micrograms thyroxine in 0.1 ml alkaline saline on the 15th embryonic day. Control groups included embryos injected with thyroxine on the 15th embryonic day or 0.1 ml isotonic saline, pH 11, on both the 13th and 15th embryonic days.

Histological examination was performed on the bursa of two embryos from each experimental group on the 18th embryonic day. The remaining animals were allowed to hatch and were injected with 1 X 10^8 sheep erythrocytes in an equal volume of complete Freund's adjuvant when they were five weeks of age. Direct hemagglutination titers were determined on sera taken 6 and 12 days after antigen injection. A second injection of the same amount of antigen without adjuvant was given on day 12, and antibody titers were determined on sera taken 3, 6, and 24 days later.

This experiment was repeated with a few modifications. In the second experiment, 2.5 mg testosterone propionate in 0.1 ml sesame oil (Wollins Pharmacal Corp.) was injected into the allantoic cavity of eggs on the 12th day of incubation. On the 14th and 16th embryonic days, 0.5 ug thyroxine in 0.1 ml alkaline saline was injected into previously untreated eggs and into half of the testosterone-treated eggs. A fourth group was injected with sesame oil on the 12th embryonic day. Histological examination was performed on bursas taken from each group on the 18th embryonic day. All remaining animals were allowed to hatch and were injected with sheep erythrocytes at 5 weeks of age. A second injection of antigen was given 9 days later. All animals were bled for direct hemagglutinin titer determinations on day 5, 7, 12, 15, and 24 after the first injection of antigen.

Additional experiments were performed by varying the

dosages and embryonic day of injection of thyroxine after testosterone treatment of the embryos on day 12. Antibody response was again determined by antigen injection when the birds were five weeks of age.

A similar experimental design was used with another group of testosterone-treated embryos to test the effect of somatotropic hormone. Eggs were injected with 3.7 mg aqueous testosterone on the 11th embryonic day. The eggs were allowed to hatch and half of the birds were injected with 1 mg STH daily, throughout the experiment. These birds, along with untreated normal birds, were challenged with sheep erythrocytes when they were 4 weeks of age. Sera were taken for antibody determination on days 5, 7, and 12 after antigen injection.

Effect of Thyroxine and Somatotropic Hormone on the Immune Response

Hormone levels increased by injections of STH and thyroxine. The effects of increasing the levels of somatotropic hormone and thyroxine in normal chickens were studied. Six-week-old, adult birds were divided into four groups and treated in the following manner: 1) daily injections of thyroxine and STH throughout the experiment, 2) daily injections of thyroxine and STH for the first 16 days only, 3) daily injections of thyroxine throughout the experiment, and 4) untreated control animals. Eighteen micrograms of thyroxine in 0.1 ml alkaline saline were injected,

intramuscularly, daily for 16 days, and the dosage was then increased to 30 ug daily throughout the remainder of the experiment. Four milligrams of STH in 0.1 ml alkaline saline were injected, intramuscularly, daily throughout the experiment.

On the 16th day after beginning hormone administration, birds were injected with sheep erythrocytes and grafted with skin from a single line 96 bird. Sera were obtained for antibody titration on day 5 and 7 after injection of antigen. Skin grafts were observed for 24 days.

A similar experiment was performed using newly hatched chicks. Newly hatched chicks were injected daily with 1 mg STH and were challenged with sheep erythrocytes when three weeks of age. Sera were obtained for antibody titration on day 5, 7, and 12 after injection of antigen. The antibody titers were compared with titers of a group of untreated control chicks.

Thyroxine levels decreased by thiouracil treatment. In order to study the effects of lowering the levels of thyroxine on the immune response, the effects of thiouracil injections and feeding were studied. In the first of these experiments, 0.1 ml thiouracil suspended in sesame oil was injected into the allantoic cavity of embryonated eggs after 12 days of incubation. One, two, or four mg doses were injected into eggs in the first three groups. Three other groups of eggs received 2 ug thyroxine in addition to the

thiouracil. The number of living embryos was recorded daily after the injections and two embryos were sacrificed from each group on the 18th embryonic day for histological examination of the bursa and thyroid gland. All birds were injected with sheep erythrocytes when 5 weeks of age and sera were obtained for antibody determinations 5 and 7 days later. A second injection of sheep erythrocytes was given on day 9 and sera were again obtained 3 and 6 days later.

A second experiment involving embryonic injections of thiouracil was performed. Groups of eggs were injected with 4, 2, 1, 0.5, or 0.1 mg thiouracil suspended in 0.1 ml sesame oil on the 5th day of incubation. A group of untreated embryos was also included in the experiment. Histological examination of the bursa and thyroid of two embryos from each group was performed. When the birds were 5 weeks of age, they were injected with sheep erythrocytes and hemagglutinin titer determinations were made on sera collected 5 and 7 days later. A second antigen injection was given on day 9 and antibody titers were determined 3 and 6 days later.

The effect of thiouracil on the immune response was also studied in newly hatched chicks. This experiment involved treating groups of birds from the day of hatching in the following manner: 1) birds treated from hatching with thiouracil, 2) birds treated with thiouracil for 20 days and then thyroxine for the remainder of the experiment, 3) birds treated with thiouracil for 20 days and then thyroxine and

STH for the remainder of the experiment and, 4) untreated control birds. Thiouracil suspended in 0.1 ml sesame oil was injected in 1 mg doses for 11 days and then in 5 mg doses for the remainder of the injection schedule in each group. Nine micrograms thyroxine suspended in 0.1 ml alkaline saline were injected daily. One mg STH suspended in 0.1 ml alkaline saline was injected daily. On the 20th day after hatching, birds were injected with sheep erythrocytes and grafted with skin from a single donor. The skin donor was from a group of animals known to have B antigens foreign to the recipients. Sera were collected for antibody determinations on day 5, 7, and 12 after injection of antigen. Skin grafts were observed for 20 days.

A comparable experiment was performed with 5-week-old birds. The experimental groups included: 1) thiouracil treatment throughout the experiment, 2) thiouracil treatment for 20 days and then daily injections of thyroxine and STH and 3) untreated control animals. Two mg thiouracil suspended in 0.1 ml sesame oil were injected each day for 11 days and then 10 mg/day was injected into each bird. Eighteen ug thyroxine in 0.1 ml alkaline saline were injected daily for 20 days and then the dosage was increased to 30 ug/day. Four mg STH in 0.1 ml alkaline saline were injected daily. All animals were challenged with sheep erythrocytes and grafted with B mismatched skin 20 days after beginning experimental treatment.

Another experiment was performed by feeding thiouracil to newly hatched chicks instead of injecting it. Chicks were fed ad libitum with feed containing 0.1% (w/w) thiouracil. At 37 days of age, half of the birds in the thiouracil fed group and half of the birds in the untreated control group were sacrificed for bursa, spleen, and body weight determinations. The remainder of the birds were grafted with skin from an out of line donor. The grafts were observed for 10 days for signs of rejection. These birds were sacrificed for bursa, spleen, and body weight determinations when they were 80 days of age.

Thyroxine levels decreased by surgical thyroidectomy. Surgical thyroidectomy was performed on newly hatched chicks anesthetized with 0.03 ml Dibutol, 30 mg/ml (Sodium Pentabarbitol, Diamond Laboratories), injected intramuscularly. The thyroid was teased away from the carotid artery with opthalmic scissors and forceps. The procedure was repeated for the thyroid on both sides of the neck and the incision was closed with suture clips. Control animals were subjected to the same procedure without removal of the thyroid.

Body weights of all birds were determined at intervals throughout the experiment. At 26 days of age, all birds were injected with 0.5 ml 10% sheep erythrocyte suspension. A second injection of the same amount of sheep erythrocytes was given 20 days later. Sera for hemagglutinin titers were collected on days 3, 6, 9, 20, 23, and 26 after the first

injection of antigen. When the birds were 85 days of age, they were injected with 4×10^9 killed Salmonella organisms per kilogram body weight and bled 8 days later for bacterial agglutinin titer determinations. On day 95, all birds were sacrificed for bursa, spleen, and body weight determinations.

A second thyroidectomy experiment was also performed. Thyroidectomy was accomplished in newly hatched chicks in the manner described previously. When the birds were 20 days of age, they were injected with 0.05 ml 10% sheep erythrocyte suspension per 10 gm body weight. Serum samples were collected for hemagglutinin titer determinations 5, 7, 9, 18, and 22 days later. A second injection of 0.5 ml 10% sheep erythrocyte suspension was given 22 days after the first antigen injection. Serum samples were collected for antibody determinations 3, 6, and 10 days later. Total and differential leucocyte counts were determined when the birds were 38 days old. When the birds were 8 weeks old, they were grafted with skin from one of the control birds. Skin grafts were observed for 20 days.

STH levels decreased by antiserum to STH. The ability of anti-somatotropic hormone serum to cause histological changes in embryos or to decrease the immune response was studied. This experiment included three groups of birds: 1) birds injected with normal rabbit serum (NRS), 2) birds injected with rabbit anti-somatotropic hormone (ASTH), and 3) saline-injected control birds. Each egg was injected

in the allantoic cavity with 0.1 ml NRS, ASTH, or saline on the 9th, 11th, 13th, 15th, and 18th day of incubation. Two embryos from each group were sacrificed on the 18th embryonic day for histological examination of the bursa, thymus and spleen. Two-tenths ml ASTH, NRS, or saline were injected intravenously, daily, beginning on the 13th day after hatching and continuing throughout the experiment. Sheep erythrocytes were injected intramuscularly into each bird on the 16th day after hatching. Serum samples were collected for direct hemagglutinin titer determination 3, 6, and 9 days later.

STH levels decreased by antiserum to chicken pituitary extract. The effect of rabbit antiserum against chicken pituitary extract on the immune response was studied. Groups of birds were injected with rabbit gamma globulin directed against chicken pituitary extract (ACPE), normal rabbit globulin (NRG), or saline. Injections of 0.2 ml ACPE containing 40 mg protein per milliliter and injections of 0.3 ml NRG containing 26 mg protein per milliliter were given on days 0, 3, 6, 9, 13, and 17 after hatching. All injections were intramuscular except for the final two injections on day 13 and 17 which were intravenous.

All birds were injected intramuscularly with sheep erythrocytes on day 13 after hatching. Serum samples for hemagglutinin titration were collected 4, 7, 13, and 20 days later. Total leucocyte counts were performed on the blood

of all birds at 18 days of age.

Effect of Thyroxine and Somatotropic Hormone on Ontogeny of the Immune Response

The effect of thyroxine and STH on the development of the capacity to produce antibodies against sheep erythrocytes was studied. To determine the ontogeny of antibody formation against sheep erythrocytes in normal chicks, groups of Hyline line 96 birds were injected with 0.25 ml of a 10% sheep erythrocyte suspension divided equally into each leg on day 3, 4, 5, 6, 9, 12, or 15 after hatching. Antibody titers for each group were determined 7 days after the antigen injection. Groups of outbred White Leghorn chicks were injected on day 5, 8, 12, or 15 post-hatching with a 10% sheep erythrocyte suspension in doses of either 0.05 ml/10 g body weight or 0.5 ml per bird. Antibody titers were determined on serum samples collected 7 days after antigen injection.

Two experiments were performed to study the effect of hormones on ontogeny of the immune response. The first of these experiments contained the following groups of line 96 birds: 1) chicks injected daily with 3 ug thyroxine, 2) chicks injected daily with 1 mg STH, and 3) untreated control chicks. Injections began on the day of hatching and continued throughout the experiment. On the third day after hatching, 0.25 ml of a 10% sheep erythrocyte suspension were injected into each chick. Birds were bled for antibody

determinations on day 3, 6, and 9 after the first injection of antigen. A second injection of 0.25 ml 10% sheep erythrocytes was given 10 days after the first injection of antigen. Serum samples were taken 3 and 6 days later for antibody determinations.

In another experiment, the following 4 groups of outbred White Leghorn chicks were studied: 1) chicks injected daily with 5 ug thyroxine, 2) chicks injected daily with 1 mg STH, 3) chicks injected daily with 5 ug thyroxine and 1 mg STH and, 4) saline-injected control chicks. Injections were begun on the day of hatching and continued throughout the experiment. On day 10 after hatching, 0.5 ml of a 10% sheep erythrocyte suspension were injected into each bird. Serum samples were taken for hemagglutinin titer determinations on day 4, 7, 9, and 15 after the first injection of antigen. A second injection of sheep erythrocytes was given 15 days after the first injection of antigen. Chicks were bled 3, 7, and 11 days after the second injection of antigen for antibody determinations.

CHAPTER III

RESULTS

Effect of Thyroxine on Testosterone Treated Embryos

To test the opposing influences of testosterone and thyroxine on the embryonic immune system, effects of the following experimental treatments were studied: 1) aqueous testosterone (0.2 ml, 10 mg/ml) injected on the 13th embryonic day, 2) testosterone (as in treatment group 1), and thyroxine (2 ug in 0.1 ml alkaline saline) injected on the 15th embryonic day, 3) thyroxine (as in treatment group 2) alone, and 4) alkaline saline (0.1 ml) injected on the 13th and 15th embryonic days.

Histological examination was performed on hematoxylin and eosin stained paraffin sections of bursas and thyroids taken on the 18th day of incubation from 2 or 3 randomly chosen embryos in each experimental group. Bursas from birds injected with saline had normal lymphoid follicle development (Fig. 1). Testosterone treatment caused absence of all lymphoid differentiation (Fig. 2). Bursas from embryos treated with testosterone, followed by thyroxine, contained a few poorly developed lymphoid follicles (Fig. 3).

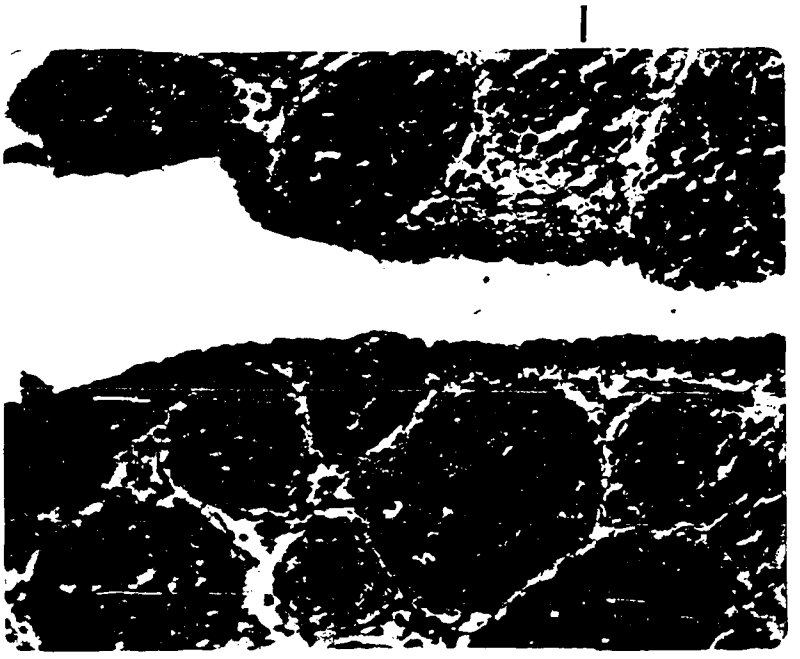


Fig. 1.--Hematoxylin and eosin stained section of bursa tissue from an 18-day-old embryo injected with saline on the 13th and 15th days of incubation (430 X).

Fig. 2.--Hematoxylin and eosin stained section of bursa tissue from an 18-day-old embryo injected with testosterone on the 13th day of incubation (430 X).

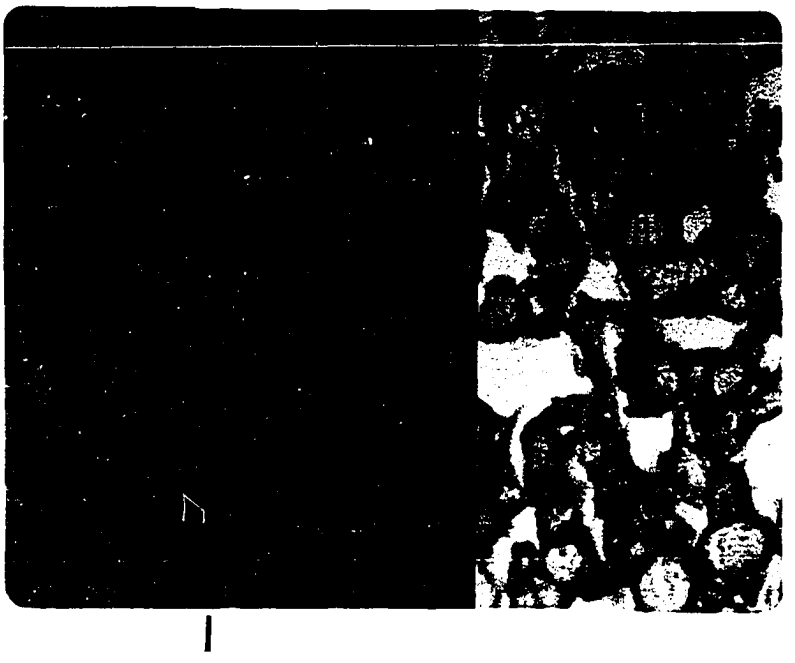
In Fig. 3, the best lymphoid development of the sections examined from this treatment group can be seen. At the level of sensitivity of this study, thyroxine alone did not alter normal bursa development. These observations confirmed the notion that testosterone and thyroxine have opposing influences on central lymphoid development, and that these influences are demonstrable histologically. They do not indicate, however, whether one or multiple mechanisms are functional, and they do not show that these influences are physiological.

Histological examination of the thyroid glands of testosterone-treated embryos revealed differences as compared to those of normal 18-day-old embryos. The thyroids from testosterone-treated embryos appeared hyperplastic and hypertrophic. In addition, mean follicle diameters were smaller and an apparent change in colloid consistency occurred. Thyroid sections from both groups are shown in Fig. 4. The mean diameters of 100 follicles for normal and testosterone-treated embryos are presented in Table 1. The effect of thiouracil treatment is included for reference since it is known that thiouracil inhibits thyroxine synthesis (134).

The birds which were allowed to hatch were injected with 10^8 sheep erythrocytes in complete Freund's adjuvant when they reached 5 weeks of age. A second dose of 10^8 sheep erythrocytes, given without adjuvant, was injected 12 days

Fig. 3.--Hematoxylin and eosin stained section of bursa tissue from an 18-day-old embryo injected with testosterone on the 13th day of incubation followed by thyroxine injection on the 15th day of incubation (300 X).

A



B

Fig. 4.--Hematoxylin and eosin stained section of thyroid tissue from an 18-day-old embryo injected with A) saline or B) testosterone (430 X).

TABLE 1

EFFECT OF TESTOSTERONE TREATMENT ON EMBRYONIC THYROID FOLLICLE DIAMETER

Experimental Treatment	Mean Follicle Diameter[a]
Saline	17.7±5.1
Testosterone	15.9±5.2[b]
Thiouracil	14.5±6.2[b]

[a]Mean follicle diameter in microns ± 1 standard deviation from the mean.

[b]$p=.02$ as compared to the saline group.

later. The mean $\log_2$ antibody titers of these experimental groups is shown in Table 2. The testosterone-treated group could be divided into subgroups on the basis of antibody forming capability. Four of six birds had maximum antibody titers of less than 3 and are shown separately in Fig. 5. Their titers are included in the mean for the entire testosterone group.

Because some of the titers from animals in the testosterone and the testosterone and thyroxine treated groups were inordinately high, serum samples were fractionated by ultracentrifugation. Antibody titers were determined on the fractions to see if these animals had relatively larger amounts of antibody in the heavy portion of the gradient. The direct and indirect titers of these fractions are shown in Fig. 6.

The experiment was repeated with several modifications. The experimental groups were as follows: 1) testosterone propionate (TP) (2.5 mg in 0.1 ml sesame oil), injected on the 12th embryonic day, 2) TP (as in treatment group 1) and thyroxine (0.5 ug in 0.1 ml alkaline saline) on the 14th and 16th embryonic days, 3) thyroxine (as in treatment group 2) alone, and 4) sesame oil (0.1 ml) injected on the 12th embryonic day for the control group.

On histological examination, the bursas of each group appeared similar to the bursas from corresponding groups in the previous experiment. However, thyroxine treatment after

TABLE 2

ANTIBODY FORMING CAPACITY OF BIRDS TREATED WITH TESTOSTERONE ON THE 13TH EMBRYONIC DAY FOLLOWED WITH THYROXINE ON THE 15TH EMBRYONIC DAY

Experimental Treatment	No. of Animals	Days After Antigen Injection[a]				
		6	12	15	18	36
Testosterone	6	0	0.1±0.4	3.7±4.4[b]	7.5±9.8[c]	8.1±9.1
Nonresponders[d]	4	0	0	1.3±0.5[e]	2.0±0.5[e]	2.2±0.5
Testosterone and Thyroxine	4	0	0.8±1.3	8.2±1.3[f]	18.8±3.8[f]	22.3±4.0[f]
Thyroxine	5	1.2±1.1	8.0±3.1	9.2±3.5	9.6±2.3	10.2±1.8
Saline	2	0	10.0±2.0	10.5±4.9	11.5±3.5	12.1±2.8

[a] A second injection of antigen was given on day 12.

[b] Mean Log_2 antibody titer ± 1 standard deviation from the mean.

[c] $p=0.05$, compared with saline.

[d] Testosterone-treated birds whose peak antibody titer was less than 3.0.

[e] $p=0.005$, compared with saline.

[f] $p=.01$, compared with 4 testosterone nonresponders.

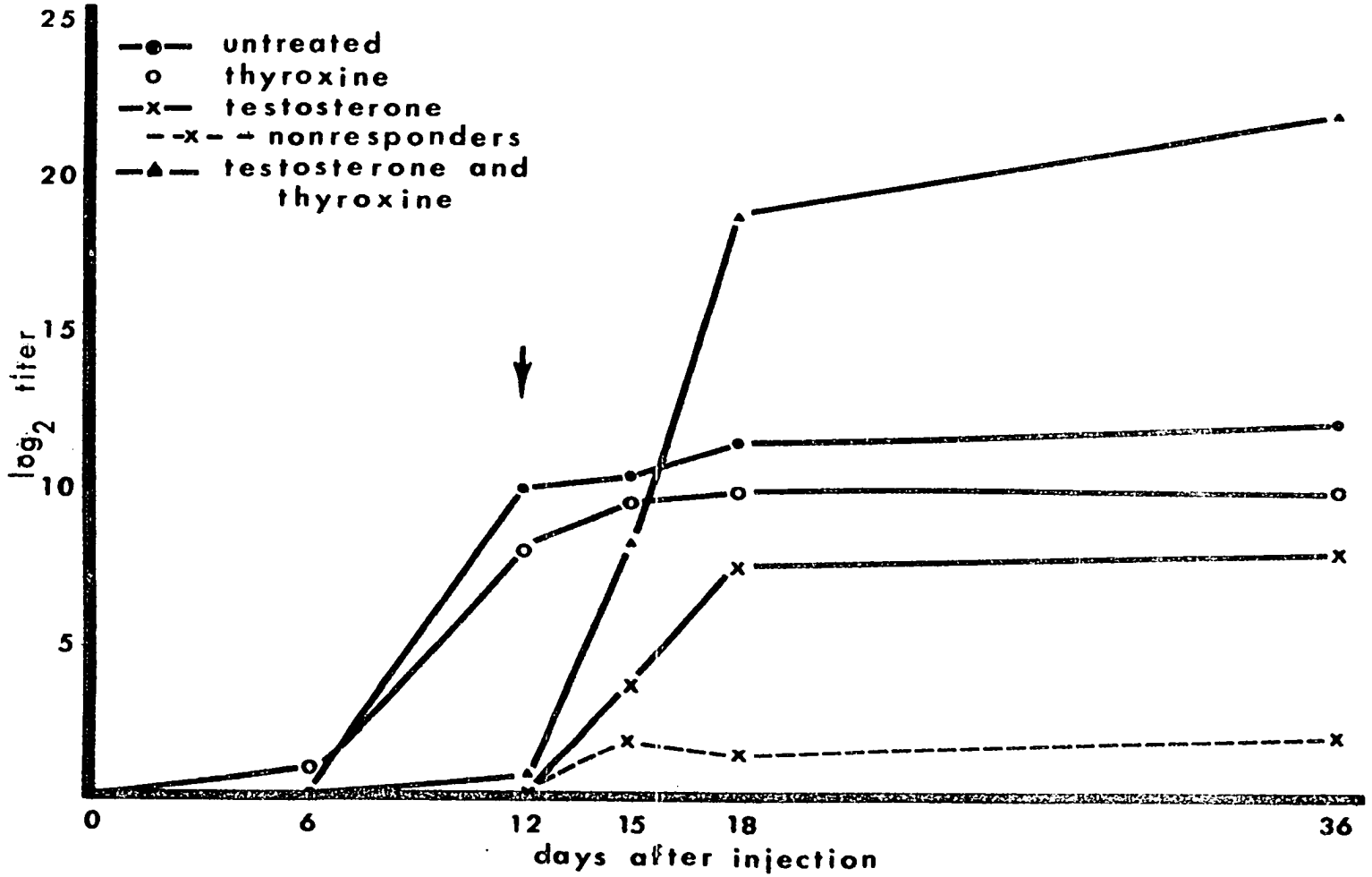


Fig. 5.--Anti-sheep erythrocyte response of five-week-old birds treated with testosterone on the 13th embryonic day followed by thyroxine on the 15th embryonic day. Arrow indicates a second injection of antigen.

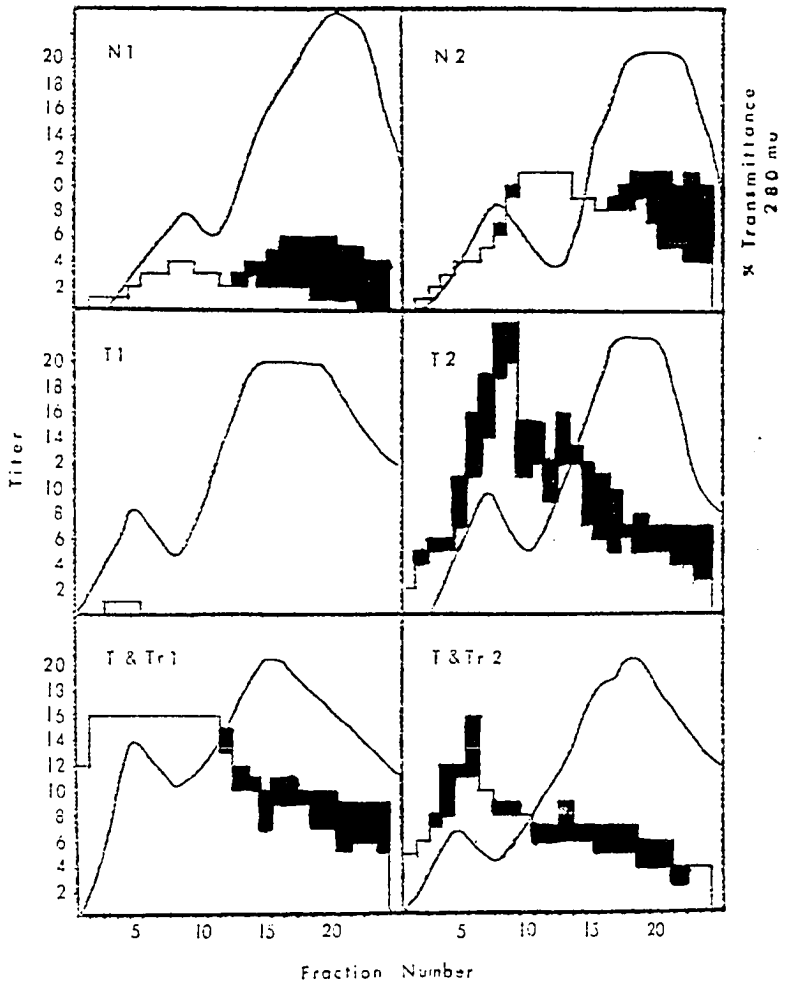


Fig. 6.--Mean log2 direct (clear bars) and indirect (black bars) hemagglutinin titers of ultracentrifuge fractions of sera taken 6 days after a second injection of antigen. The sera was taken from normal (N), testosterone (T), or testosterone and thyroxine (T & Tr) treated animals. Direction of sedimentation is right to left.

TP treatment did not increase the lymphoid cellularity in the embryonic bursa. Thyroid glands were not available for evaluation.

When the birds were 5 weeks of age, 0.5 ml 10% sheep erythrocytes were injected without adjuvant. A second dose of antigen was injected 9 days later. The antibody response of these experimental groups is presented in Fig. 7. The TP treated birds did not respond well to either a first or second injection of antigen. Antibody titers of sera from the TP group were significantly different from those of the control birds ($p=.01$). The TP and thyroxine-treated group had higher titers than did the group treated with TP alone, but this group was not significantly different when compared with either normal controls or the TP group. The individual titers within these groups are presented in Table 3. Bimodal distribution of titers is evident in both the TP and thyroxine group. The mean titer of the responders ($\log_2$ titer greater than 2) in the TP and thyroxine group is significantly different from the testosterone group ($p=.01$).

Another experiment was designed along similar lines to test the effects of larger doses of thyroxine during the embryonic period. Sheep erythrocytes were injected into all animals when they were 5 weeks of age. A second injection of antigen was given 9 days later. In this experiment, testosterone propionate failed to produce a clear hormonal bursectomy in most of the animals. Antibody titers of the TP group

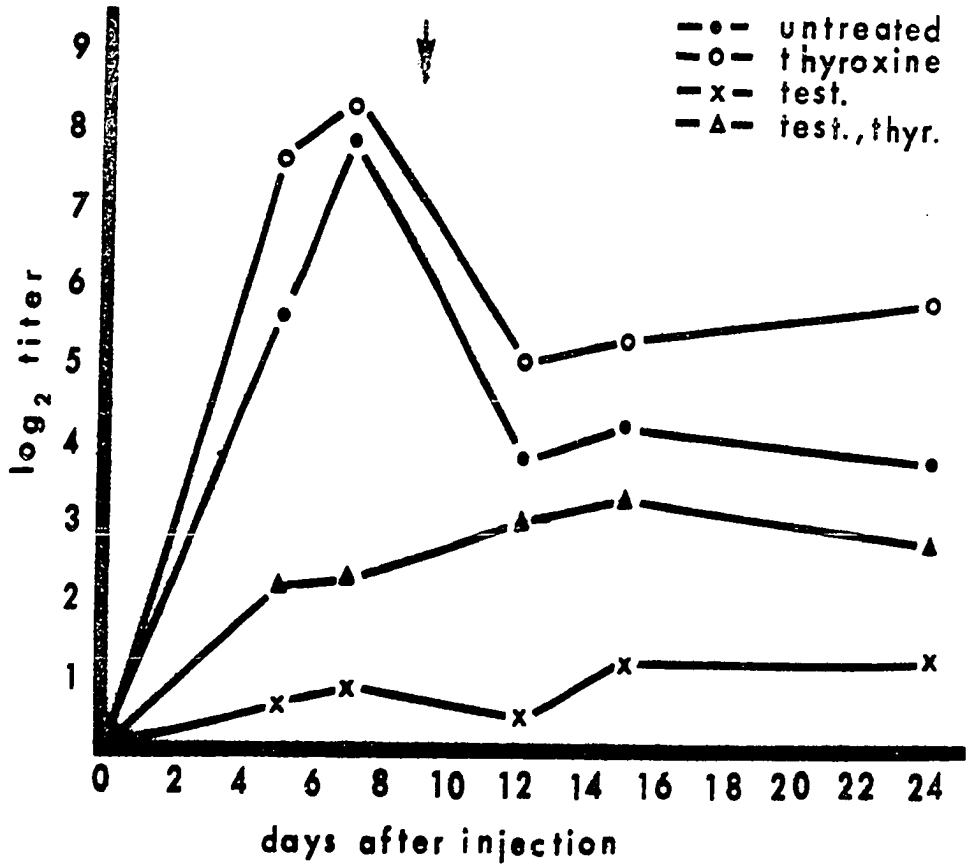


Fig. 7.--Anti-sheep erythrocyte response of five-week-old birds treated with testosterone on the 12th embryonic day followed by thyroxine on the 14th and 16th embryonic days. Arrow indicates a second injection of antigen.

TABLE 3

INDIVIDUAL TITERS OF SERA TAKEN SIX DAYS AFTER A SECOND INJECTION OF ANTIGEN

Experimental Treatment	Individual Log_2 Titers
Control	3.5, 3.5, 4, 6
Thyroxine	9, 4.5, 4.5, 3
Testosterone Propionate and Thyroxine	10, 4, 3.5, 2, 0, 0
Testosterone Propionate	4, 1, 0, 0

were lower than those found in control animals during the peak primary immune response. The experimental treatments and the antibody titers are presented in Table 4. The lower titers in some of the groups treated with thyroxine may show that thyroxine has a detrimental effect on antibody formation if doses are given at higher than optimum levels or are given at very early embryonic age.

A similar experimental design was used to test the ability of STH to influence antibody production after hormonal bursectomy with testosterone. Hormonal bursectomy was accomplished by injecting 3.7 mg aqueous testosterone into eggs on the 11th day of incubation. The chicks were allowed to hatch and some of them were treated with daily injections of STH. A third group (untreated as embryos) was included for comparison. At 5 weeks of age, all birds were injected with sheep erythrocytes. The antibody titers are presented in Table 5. Hormonally bursectomized birds treated with STH did not show greater antibody forming capacity than hormonally bursectomized birds not receiving STH. A slight difference, however, in survival potential was noted. Four of 7 testosterone-treated birds were alive at 5 weeks of age while all of 5 testosterone-treated birds given daily injections of STH were alive at that time.

TABLE 4

ANTIBODY FORMING CAPACITY OF BIRDS TREATED EMBRYONICALLY WITH TESTOSTERONE AND VARYING AMOUNTS OF THYROXINE

Treatment	No. of Animals	Days After Antigen Injection[a]			
		5	7	12	15
1. TP(12)[b]	9	5.3±1.4[c]	*5.9±2.5	***9.2±2.3	9.8±1.5
2. TP(12) Tr(12, 15)[d]	2	3.0±4.2	*3.5±2.1	4.0±4.2	5.3±5.3
3. Tr(12, 15)	5	4.0±2.2	*5.4±3.2	5.7±3.8	7.6±1.2
4. TP(12) Tr(12, 15)[e]	5	**2.6±1.3	**1.7±1.1	5.8±3.7	6.0±3.7
5. Controls	7	5.9±0.7	8.1±0.6	6.0±1.6	7.3±1.8

*p=.005 as compared to controls.

**p=.001 as compared to controls.

***p=.025 as compared to controls.

[a]A second injection of sheep erythrocytes was given on day 9.

[b]Testosterone propionate was injected in 2.5 mg amounts on embryonic day 12.

[c]The numbers are presented as mean $\log_2$ titers ± 1 standard deviation from the mean.

[d]Thyroxine was injected into group 2 and 3 in amounts of 0.5 ug on embryonic day 12 and 2 ug on day 15.

[e]Group 4 received thyroxine on embryonic day 12 and 15 in amounts of 0.5 ug and 1.0 ug.

TABLE 5

EFFECT OF DAILY STH INJECTIONS ON ANTIBODY PRODUCTION IN HORMONALLY BURSECTOMIZED BIRDS

Treatment	No. of Animals	Day after Antigen Injection		
		5	7	12
Testosterone	4	1.0[a]	0.8	3.5
Testosterone + STH[b]	5	1.2	2.0	3.8
Control	5	8.4	7.2	8.9

[a]Mean $\log_2$ anti-sheep erythrocyte titers.

[b]Somatotropic hormone.

Effects of Thyroxine and Somatotropic Hormone on the Immune Response

Increased Levels of Hormones

Groups of adult line 96 birds were injected daily with: 1) thyroxine (18 ug in 0.1 ml for 16 days, then 30 ug in 0.1 ml) and STH (4 mg in 0.1 ml) throughout the experiment, 2) thyroxine (18 ug in 0.1 ml) and STH (4 mg in 0.1 ml) for the first 16 days only, 3) thyroxine (as in treatment group 1) throughout the experiment, or 4) saline (0.1 ml).

The antibody titers in the experimental groups did not differ significantly from the titers in the control group (Table 6). Skin graft rejection time was not altered by any of the hormonal treatments (Table 7). Skin grafts used in this experiment were antigenic for the recipients only by minor histocompatibility antigens in order to detect any shortened survival times due to increased hormone levels. Data are presented as the cumulative number of grafts rejected in each group at different times after grafting.

The influence of STH on the antibody forming capacity of newly hatched chicks was studied. STH (1 mg in 0.1 ml) was injected daily into chicks and at 3 weeks of age the chicks were challenged with sheep erythrocytes. No significant alteration in antibody production was noted (Table 8).

Decreased Levels of Hormones

Thiouracil. The effect of injection of thiouracil (TU) into embryos on the 12th day of incubation was studied.

TABLE 6

EFFECT OF STH AND THYROXINE ON ANTIBODY PRODUCTION IN ADULT BIRDS

Treatment	No. of Animals	Day After Antigen Injection[a]	
		5	7
Tr & STH[b]	3	3.2[c]	6.7
Tr & STH/Stopped[d]	3	4.3	4.0
Thyroxine[e]	2	1.5	4.3
Control	5	2.7	4.5

[a]Sheep erythrocytes injected 16 days after beginning hormone treatments.

[b]Daily thyroxine and somatotropic hormone injections throughout the experiment.

[c]Mean $\log_2$ anti-sheep erythrocyte titer.

[d]Daily thyroxine and STH injections for the first 16 days of the experiment.

[e]Daily thyroxine injections throughout the experiment.

TABLE 7

EFFECT OF STH AND THYROXINE ON GRAFT REJECTION TIMES IN ADULT BIRDS

Treatment	No. of Animals	Day After Grafting[a]		
		10	12	24
Tr & STH[b]	3	1[c]	1	1
Tr & STH/Stopped[d]	3	0	1	1
Thyroxine[e]	2	0	0	0
Controls	5	0	1	1

[a]Skin was grafted to all birds 16 days after beginning hormone treatments.

[b]Daily thyroxine and somatotropic hormone injections throughout the experiment.

[c]Cumulative number of grafts rejected.

[d]Daily thyroxine and STH injections for the first 16 days of the experiment.

[e]Daily thyroxine injections throughout the experiment.

TABLE 8

EFFECT OF DAILY STH INJECTIONS ON ANTIBODY PRODUCING CAPACITY IN THREE-WEEK-OLD CHICKENS

Treatment	No. of Animals	Day After Antigen Injection		
		5	7	12
STH	10	6.2[a]	6.3	8.1
Control	10	8.4	7.2	8.9

[a]Mean $\log_2$ anti-sheep erythrocyte titer.

Groups of eggs were injected into the allantoic cavity with 0.1 ml of varying concentrations (see Table 9) of TU suspended in sesame oil. The chicks were allowed to hatch, and their antibody response to sheep erythrocytes was evaluated when they were 5 weeks old. A second injection of antigen was given 9 days after primary immunization. The survival of the chicks and their antibody responses are shown in Table 9. No significant effect of thiouracil injected on embryonic day 12 was noted.

In order to produce an earlier blockage of thyroxine production in embryonic life, a similar experiment was performed injecting thiouracil on the 5th day of incubation. The birds were allowed to hatch and sheep erythrocytes were injected when they were 5 weeks old. A second dose of antigen was injected 9 days after primary immunization. Antibody titers of birds treated on the 5th embryonic day with varying concentrations of TU are presented in Table 10. No significant effect of this treatment on antibody production was noted.

Histological examination on embryonic day 18 revealed hyperplastic and hypertrophic thyroids, indicating probable hypofunction of the thyroid gland due to thiouracil treatments. Bursa morphology on embryonic day 18 in both experiments was indistinguishable from controls (Fig. 8).

The effect of thiouracil was next studied in newly hatched chicks. Antibody formation and skin graft rejection

TABLE 9

EFFECT OF THIOURACIL INJECTIONS ON EMBRYONIC DAY 12 ON THE VIABILITY AND ANTIBODY FORMING CAPACITY OF FIVE-WEEK-OLD CHICKENS

Treatment	No. Animals Injected	No. Animals at 5 Weeks	Day after Antigen Injection[a]			
			5	7	12	15
TU (1mg)	10	6	6.5±2.6[b]	7.9±2.5	5.8±3.1	6.0±2.3
TU (2mg)	10	8	6.9±2.2	8.5±2.0	8.4±3.3	7.7±2.3
TU (4mg)	10	4	5.9±3.6	5.2±4.1	6.5±0.7	7.5±1.1
Control	-	5	7.7±1.9	8.1±1.2	7.8±1.0	8.8±2.0

[a]A second injection of antigen was given on day 9 after primary immunization.

[b]Mean $\log_2$ anti-sheep erythrocyte titers ± 1 standard deviation from the mean.

TABLE 10

EFFECT OF THIOURACIL INJECTIONS ON EMBRYONIC DAY 5 ON THE VIABILITY AND ANTIBODY FORMING CAPACITY OF FIVE-WEEK-OLD CHICKENS

Treatment	No. Inj.	No. at 5 wks.	Day After Antigen Injection[a] 5	7	12	15
TU (4mg)	8	2	6.0[b]	7.3	6.0	5.0
TU (2mg)	8	5	5.9	7.3	6.6	7.6
TU (1mg)	8	2	6.8	7.5	6.8	5.5
TU (.5mg)	8	8	6.4	7.5	8.3	8.1
TU (.1mg)	8	8	5.4	5.3	5.9	8.7
Control	-	7	5.9	8.1	6.0	7.3

[a]A second injection of antigen was given on day 9 after primary immunization.

[b]Mean $\log_2$ anti-sheep erythrocyte titers.

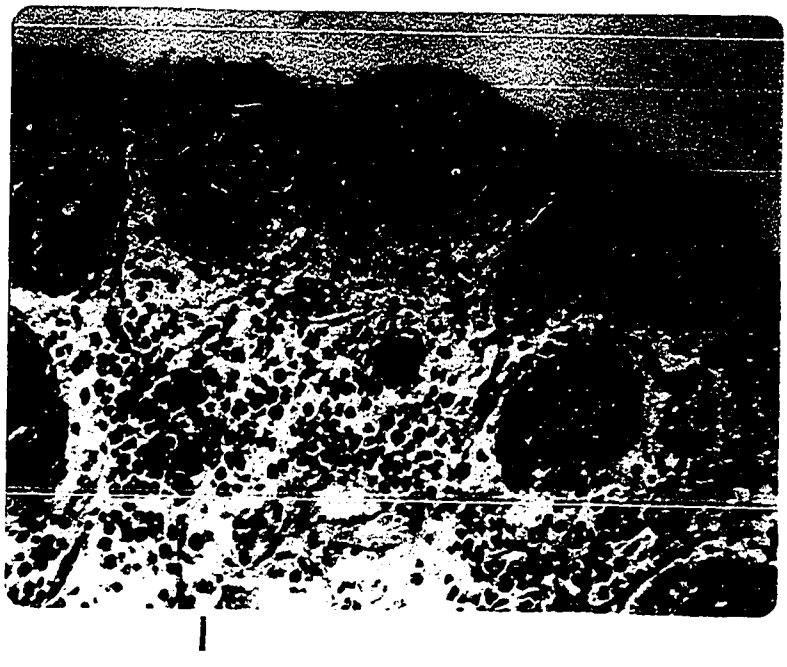


Fig. 8.--Hematoxylin and eosin stained section of bursa tissue from an 18-day-old embryo treated with thiouracil on the 5th embryonic day (430 X).

were studied in the following experimental groups of newly hatched chicks: 1) daily 0.1 ml thiouracil injections (1 mg daily for the first 11 days, then 5 mg daily for the remainder of the experiment), 2) daily thiouracil injections (1 mg increased to 5 mg as in treatment group 1) for 20 days and then thyroxine (9 ug in 0.1 ml alkaline saline) daily for the remainder of the experiment, 3) thiouracil (as in treatment group 2) for 20 days followed by thyroxine (as in treatment group 2) and STH (1 mg daily) for the remainder of the experiment and 4) untreated control birds.

Sheep erythrocytes were injected and skin was grafted 20 days after beginning treatment. Again, thiouracil did not alter antibody production (Table 11). Mean graft rejection times were not different in the normal and experimental groups (Table 12). A slightly slower rate of rejection might be suggested in birds treated with thiouracil throughout the experiment. However, autopsy of birds in this experiment revealed no overt alteration of thyroid morphology by the thiouracil treatment.

A similar experiment was performed using adult birds. Birds 5 weeks of age were treated as follows: 1) daily thiouracil injections (2 mg/day for 11 days, then 10 mg/day), 2) daily injections of TU (as in treatment group 1) for 20 days and then daily injections of thyroxine (18 ug/day for 20 days, then 30 ug/day) and STH (4 mg/day) for the remainder of the experiment, and 3) untreated control birds. The

TABLE 11

EFFECT OF DAILY THIOURACIL INJECTIONS ON ANTIBODY PRODUCTION IN 20-DAY-OLD CHICKENS

Treatment	No. of Animals	Day After Antigen Injection		
		5	7	12
TU[a]	6	6.6[b]	8.2	8.0
TU/Tr[c]	6	6.7	7.3	8.0
TU/Tr STH[d]	6	5.0	5.8	8.6
Control	10	6.0	6.9	8.4

[a]Daily thiouracil injections from the day of hatching.

[b]Mean $\log_2$ anti-sheep erythrocyte titer.

[c]Daily thiouracil injections until day 20, then daily thyroxine injections.

[d]Daily thiouracil injections until day 20, then daily thyroxine and somatotropic hormone injections.

TABLE 12

EFFECT OF DAILY THIOURACIL INJECTIONS ON GRAFT REJECTION TIME OF 20-DAY-OLD CHICKENS

Treatment	No. of Birds	% Rejection on Day 11	Mean Rejection Time in Days
TU[a]	6	0	15.0
TU/Tr[b]	6	33	12.0
TU/Tr STH[c]	6	66	11.5
Control	10	30	12.4

[a]Daily thiouracil injections from the day of hatching.

[b]Daily thiouracil injections until day 20, then daily thyroxine injections.

[c]Daily thiouracil injections until day 20, then daily thyroxine and STH injections.

inability of thiouracil to alter the response to sheep erythrocytes and skin grafts was similar to that found in newly hatched chicks. These results are presented in Tables 13 and 14. Again, no evidence was found for alteration of thyroid function by thiouracil injections. From these same data, it is evident that injection of STH and thyroxine did not affect the responses studied.

In a third experiment, thiouracil was fed to chicks from the time of hatching. Some of these birds were sacrificed with controls on 37, 68, and 80 days of age for determination of bursa, spleen and body weights. Ten thiouracil fed and 10 control birds were injected with sheep erythrocytes on day 37. Five birds from each group were grafted with skin on day 68.

The absolute and relative (organ weight/body weight) organ weights of the thiouracil fed birds are presented as a percentage of comparable normal control values in Fig. 9. At 37 days of age, all relative and absolute organ weights of the thiouracil fed birds were significantly lower ($p=.01$) than the control weights. The thiouracil fed group included 3 males and 9 females. The control group included 2 males and 7 females. The number of animals in each group and the more even sex distribution allows a more valid comparison at 37 days of age than at the two other times. At 68 days, each group had 5 birds, but the thiouracil fed group had 5 males compared to 2 males in the normal group. At 80 days of age

TABLE 13

EFFECT OF DAILY THIOURACIL INJECTIONS ON ANTIBODY PRODUCTION OF ADULT CHICKENS

Treatment	No. of Animals	Day After Antigen Injection	
		5	7
TU[a]	6	3.4[b]	4.1
TU/STH Tr[c]	6	3.3	5.5
Control	6	3.6	3.7

[a]Daily thiouracil injections throughout the experiment.

[b]Mean $\log_2$ anti-sheep erythrocyte titer.

[c]Daily thiouracil injections for 20 days, then daily STH and thyroxine injections for the remainder of the experiment.

TABLE 14

EFFECT OF DAILY THIOURACIL INJECTIONS ON GRAFT REJECTION TIME OF ADULT CHICKENS

Treatment	No. of Animals	% Rejection on Day 13	Mean Rejection Time in Days
TU[a]	6	25	14.0
TU/STH Tr[b]	6	0	14.4
Control	6	40	13.4

[a]Daily thiouracil injections throughout the experiment.

[b]Daily thiouracil injections for 20 days, then daily STH and thyroxine injections for the remainder of the experiment.

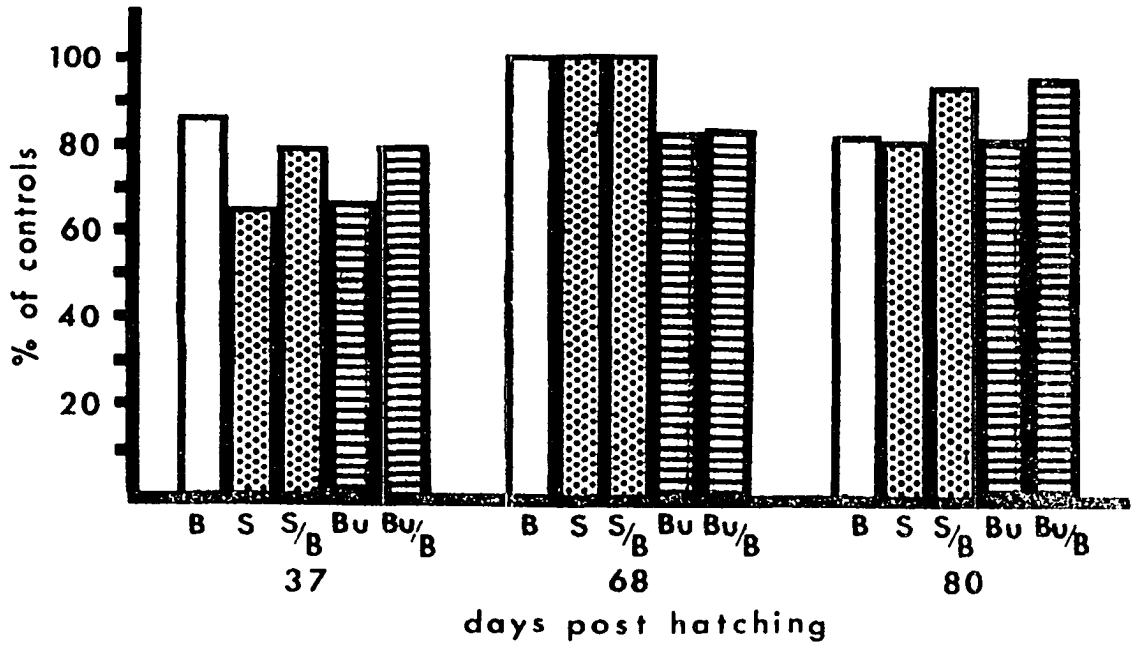


Fig. 9.--Spleen, bursa, and body weights of birds fed thiouracil from the time of hatching. Body (B), spleen (S), bursa (Bu) weights.

5 birds in each group were also studied. All were males.

The antibody response of the birds is shown in Fig. 10. Antibody titers were not significantly different at any time. The rates of decline of the titers between days 9 and 23 seem different in the thiouracil fed birds as compared to normal birds, however, this could not be confirmed statistically.

In this experiment, B mismatched skin grafts were used in order to more easily observe a prolonged survival time due to thiouracil treatment. Skin grafts were rejected in both groups by day 8 when the bandages were removed. Fifty per cent of the autografts were healthy at the same time.

In this experiment, the mean thyroid weight of the thiouracil fed birds was 16.5 times larger than that of the normal control group at 37 days of age. At 68 and 80 days, the thyroid weights were 13.8 and 13.2 times larger, respectively, than those of the control group. This indicated a hypothyroid condition, the degree of which became less severe as the birds aged.

Thyroidectomy. In order to insure a more nearly hypothyroid condition, surgical thyroidectomy was performed in newly hatched chicks. The body weights of thyroidectomized and control birds throughout the experiment are presented in Fig. 11. The weights of the six most visibly affected birds are included as a separate group. The mean

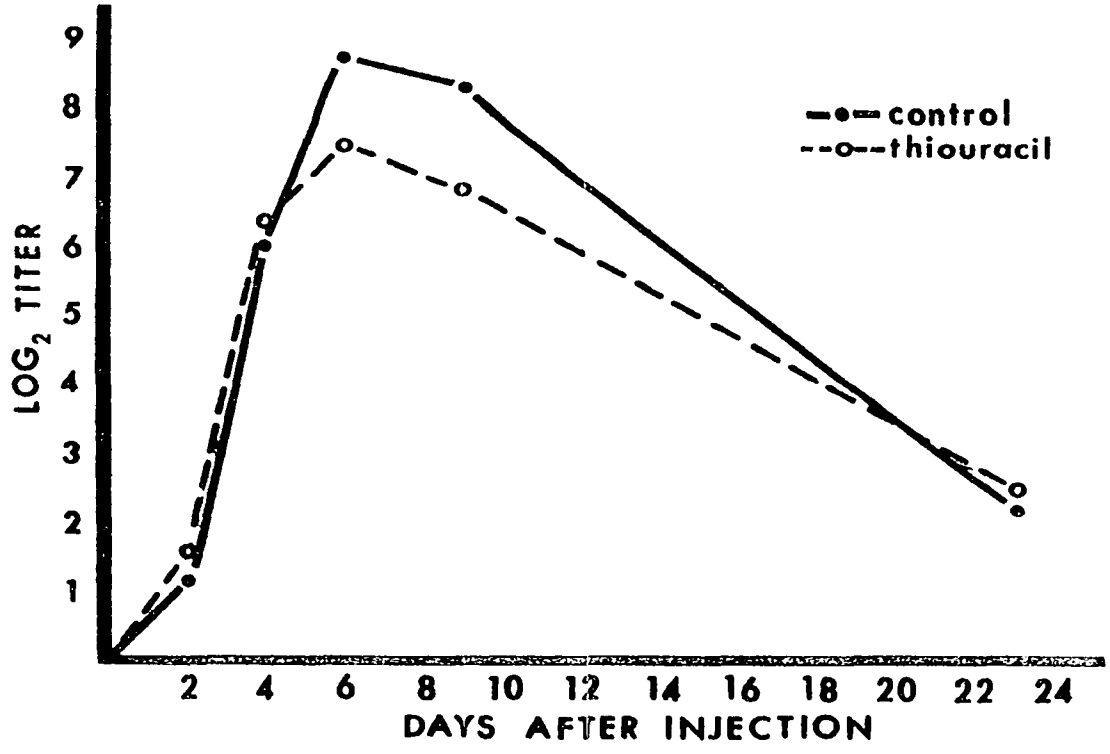


Fig. 10.--Anti-sheep erythrocyte response of 37-day-old birds fed thiouracil from the time of hatching.

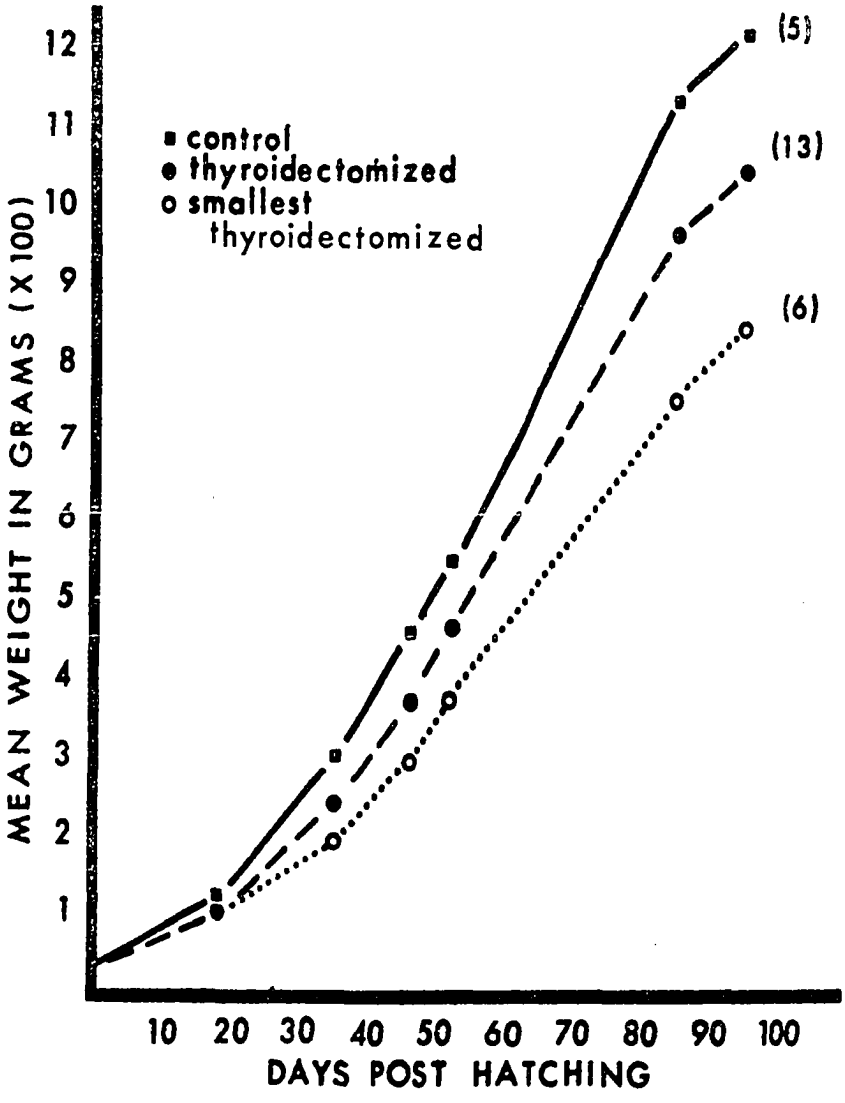


Fig. 11.--Growth rate of thyroidectomized and normal birds. The number of animals in each group is indicated by the number in ( ). The 6 smallest thyroidectomized birds are included in the thyroidectomized group containing 13 birds.

weight of the entire experimental group was not significantly different from that of the control group. The thyroidectomized group did, however, contain relatively more males than did the control group: 9 of 13, compared to 3 of 5 for the controls. This bias could easily mask the effect of thyroidectomy on the growth rate since males are normally larger than females.

The range in size and weight for all the birds at 95 days of age is indicated in Fig. 12. Some of the most severely affected thyroidectomized birds are shown with a normal bird of the same age in Fig. 13.

The most visibly affected birds in the thyroidectomized group showed an abnormality in feather growth, with the long, fringed, lacy feathers characteristic of hypothyroid birds (16). They also had reduced comb size which also is characteristic of hypothyroidism (17). These observations indicated that at least half of the surgically thyroidectomized animals were hypothyroid.

Absolute and relative organ weights are presented in Fig. 14 as percentages of the normal control values. Again, because of the large range in size of the thyroidectomized birds, body and organ weights of the group as a whole were not significantly different from the weights of normal birds.

Sheep erythrocytes were injected into all birds when they reached 26 days of age. The mean antibody responses are shown in Fig. 15. The difference between the experimental

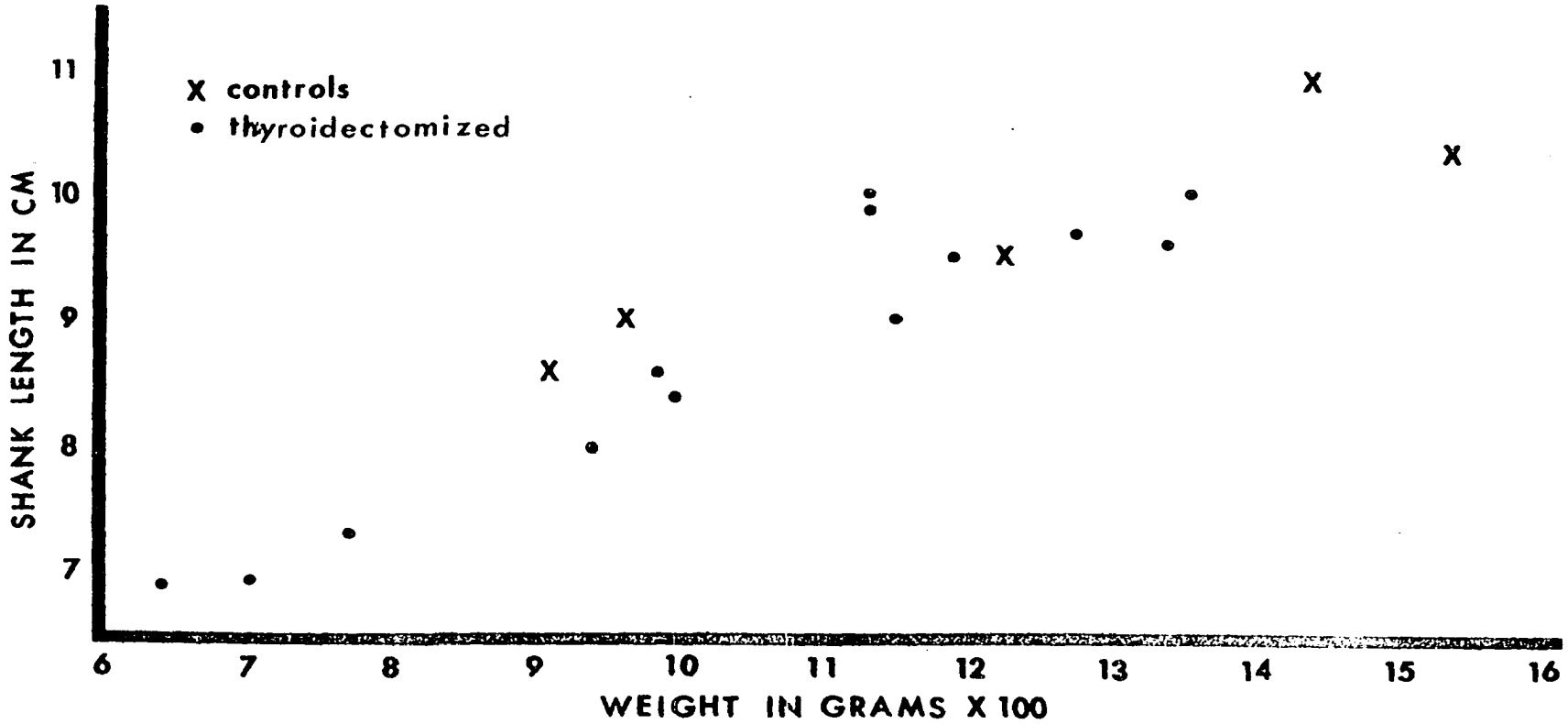


Fig. 12.--Shank length as an indication of the range in size of 95-day-old thyroidectomized and normal birds.

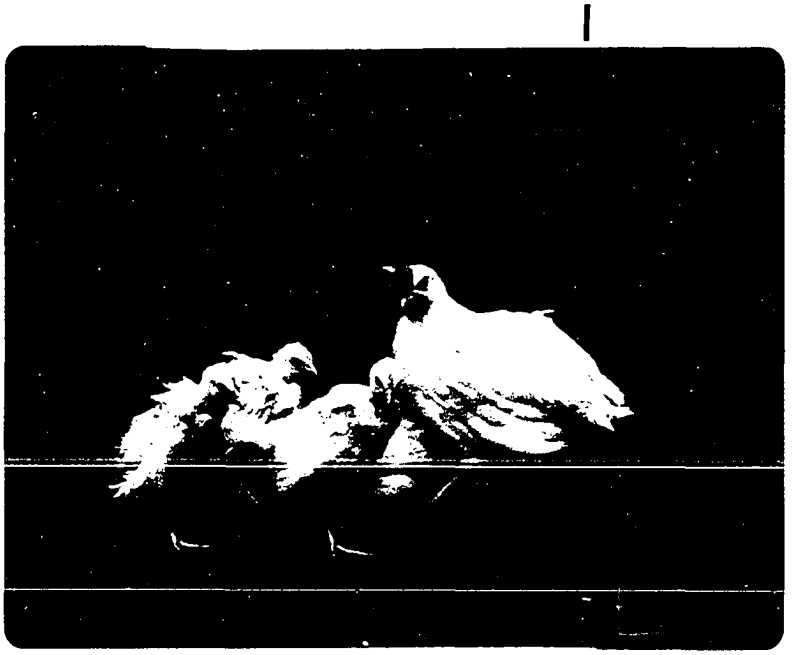


Fig. 13.--Thyroidectomized birds compared to a normal bird at 45 days of age.

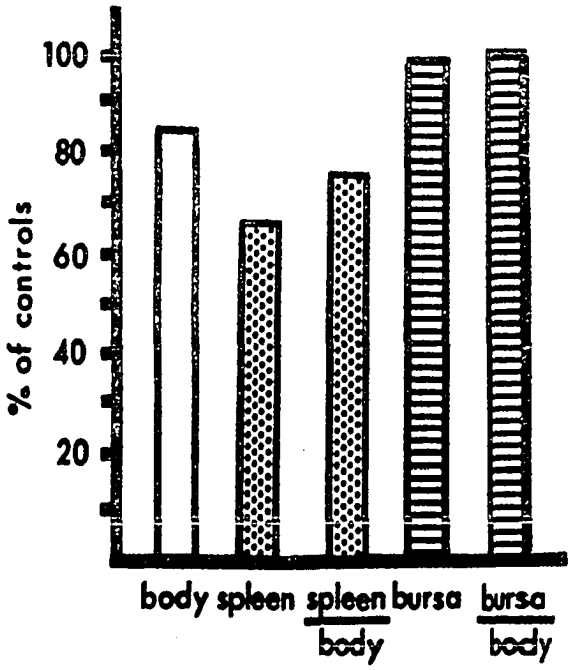


Fig. 14.--Organ and body weights of thyroidectomized birds as a percentage of control animal weights at 95 days of age.

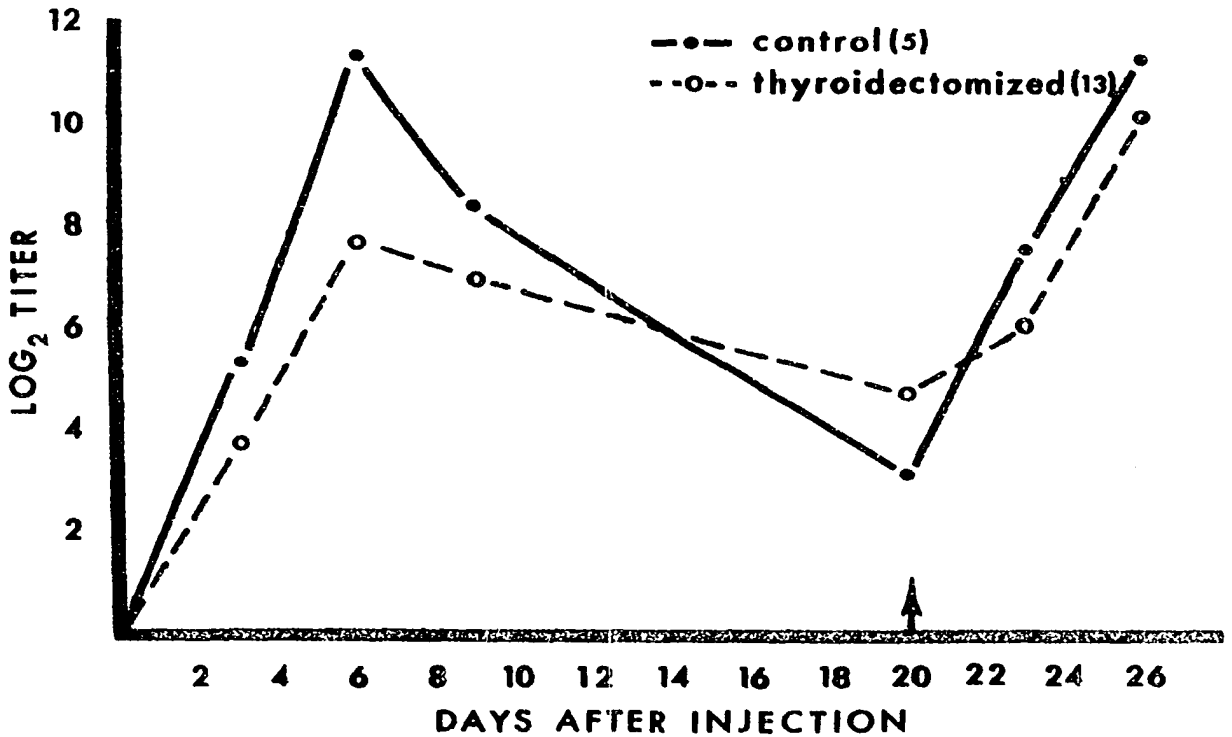


Fig. 15.--Anti-sheep erythrocyte response of 26-day-old control and thyroidectomized birds. Arrow indicates a second injection of antigen. The number of animals in each group is indicated by the number in ( ).

and control groups is significant (p=.01) on day 6. Also, the slower rate of decline in antibody titer seen in thyroidectomized birds between days 6 and 20 is statistically significant as compared to the rate of decline in normal control birds. When these birds were 85 days of age, heat killed Salmonella organisms were injected and the antibody response was determined on day 8. The antibody titer of the thyroidectomized group was again significantly lower (p=.05) than the titer of the normal animals. The individual and mean titers for both sheep erythrocytes and Salmonella organisms are shown in Fig. 16.

Since the degree of hypothyroidism might be correlated with the appearance of visible symptoms, the smallest and most severely affected birds might be expected to have lowered antibody responses. As shown in Fig. 17, no correlation can be made between body size and antibody production. The weights were determined at 32 days of age, which corresponded to the day of peak antibody titer (day 6 after sheep erythrocyte injection).

Histological examination of the lymphoid organs at 95 days of age revealed no differences in bursa morphology in the two groups of birds. The spleens of the thyroidectomized birds contained fewer lymphocytes in the thymus-dependent areas than did the spleens of control birds. The cortical areas of the thymus of thyroidectomized birds contained markedly fewer lymphocytes than did the corresponding

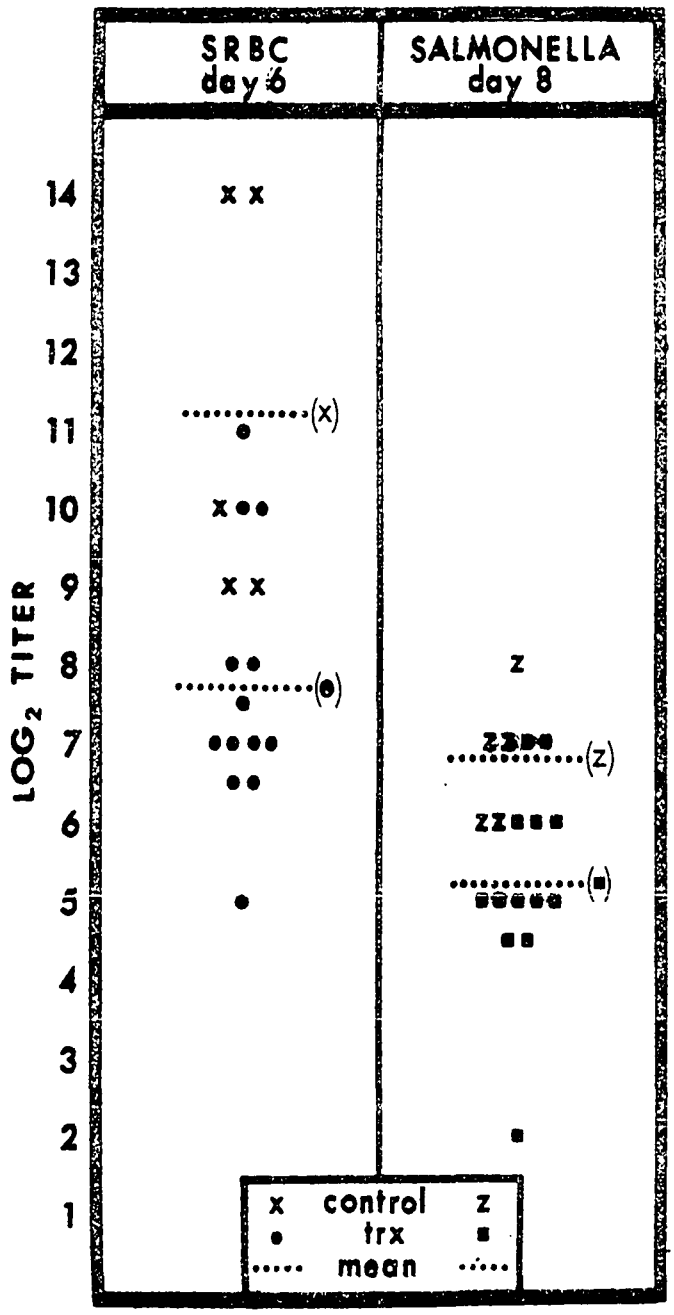


Fig. 16.--Individual peak antibody titers of control and thyroidectomized (trx) birds to sheep erythrocytes and heat-killed Salmonella organisms.

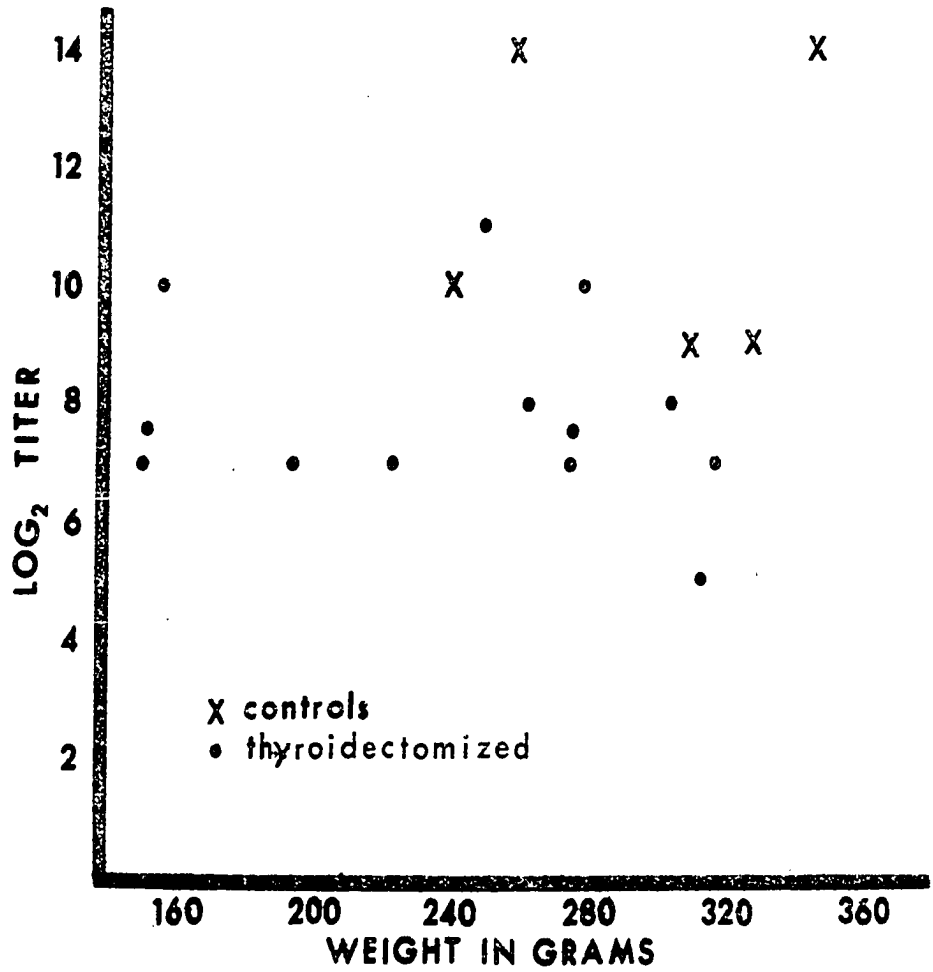


Fig. 17.--Peak anti-sheep erythrocyte titers compared to individual body weights in normal and thyroidectomized birds.

areas of thymus from control birds (Figs. 18 and 19).

A second thyroidectomy experiment was performed in order to study skin graft rejection times. Ten newly hatched chicks were thyroidectomized. Nine chicks were sham-thyroidectomized and included as a control group. The growth and appearance of the thyroidectomized birds was comparable to that seen in the previous experiment. At 20 days of age, thyroidectomized birds ranged in weight from 68 g to 124 g compared to control weights of 93 g to 169 g. The mean body weight of thyroidectomized birds was 72% of that of the normal control birds at 20 days of age and 90% at 42 days of age. Again, as in the first thyroidectomy experiment, approximately half of the thyroidectomized birds were smaller than control birds, had less feathers, and had the previously discussed, characteristic feathers. Body, bursa, and spleen weights were determined at 70 days of age. These weights are not reported because of severe weight loss and lymphoid organ atrophy related to inanition due to the accidental use of improper feeders. The larger birds, both normal control and thyroidectomized, could not eat unless the feeders were completely filled. Because of the smaller size of half of the thyroidectomized birds, they were able to eat ad libitum and continued to gain weight. This occurred after the antibody response and skin grafting experiments were completed.

When the birds were 20 days of age, sheep

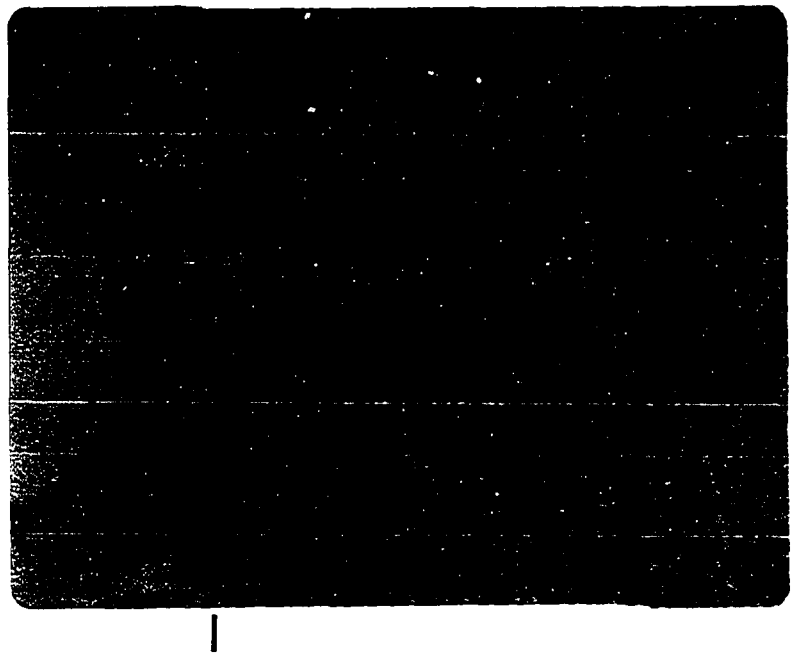


Fig. 18.--Hematoxylin and eosin stained section of thymus tissue from a normal bird at 95 days of age (430 X).

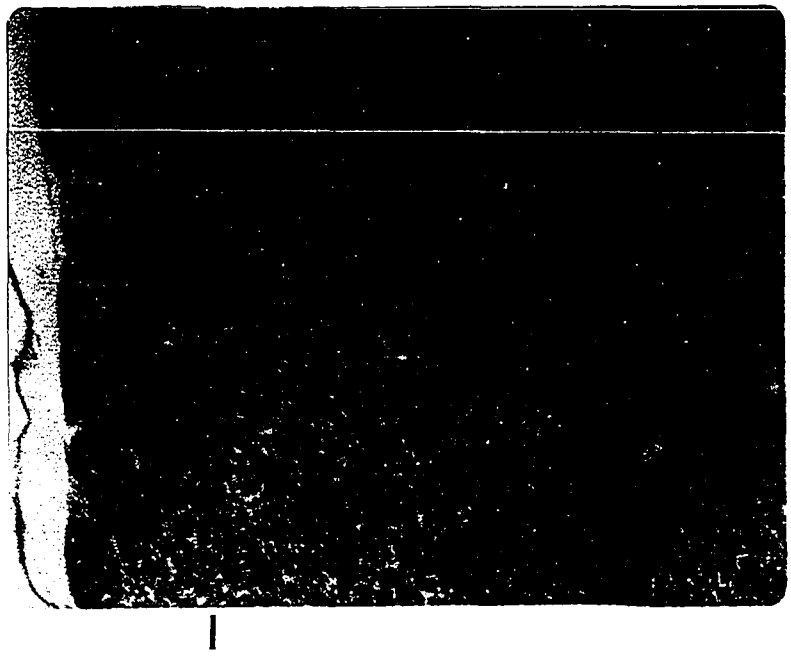


Fig. 19.--Hematoxylin and eosin stained section of thymus tissue from a thyroidectomized bird at 95 days of age (430 X).

erythrocytes (0.05 ml of a 10% suspension per 10 g body weight) were injected intramuscularly. A second dose of 0.5 ml 10% sheep erythrocytes was injected 22 days after the first antigen injection. Antibody titers are shown in Table 15. No significant differences were observed between the means of the two groups. Individual titers are shown in Fig. 20 for days 5 and 7 after antigen injection. The two highest individual titers in the thyroidectomized group were produced by the two most severely affected birds in the group.

Total leucocyte and differential counts were performed on peripheral blood from birds at 38 days of age. Total leucocyte and lymphocyte levels were reduced in thyroidectomized birds (Table 16).

At 56 days of age, skin from one of the normal control birds was grafted to each of the birds, both experimental and control. Autografts were also performed on each bird. The mean injection times of the allografts in the thyroidectomized and control group were not significantly different (Table 17). Three of the most severely affected thyroidectomized birds rejected the allografts less vigorously than did other birds. The grafts on these birds appeared smoother and did not become crusty as in typical normal graft rejections. However, they were not viable for any longer period of time than were the other grafts. In this experiment, 80% of the autografts survived.

TABLE 15

ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES OF CONTROL AND THYROIDECTOMIZED BIRDS

Treatment	No. of Animals	Day after Antigen Injection[a]								
		5	7	9	18	22	25	28	32	50
Thyroidectomy	10	6.0[b]	8.0	7.2	3.2	2.7	7.6	11.8	7.2	6.6
Control	9	8.3	9.2	6.8	2.8	2.8	7.1	8.5	6.1	6.1

[a]Second injection of sheep erythrocytes given on day 22.

[b]Mean $\log_2$ anti-sheep erythrocyte titer.

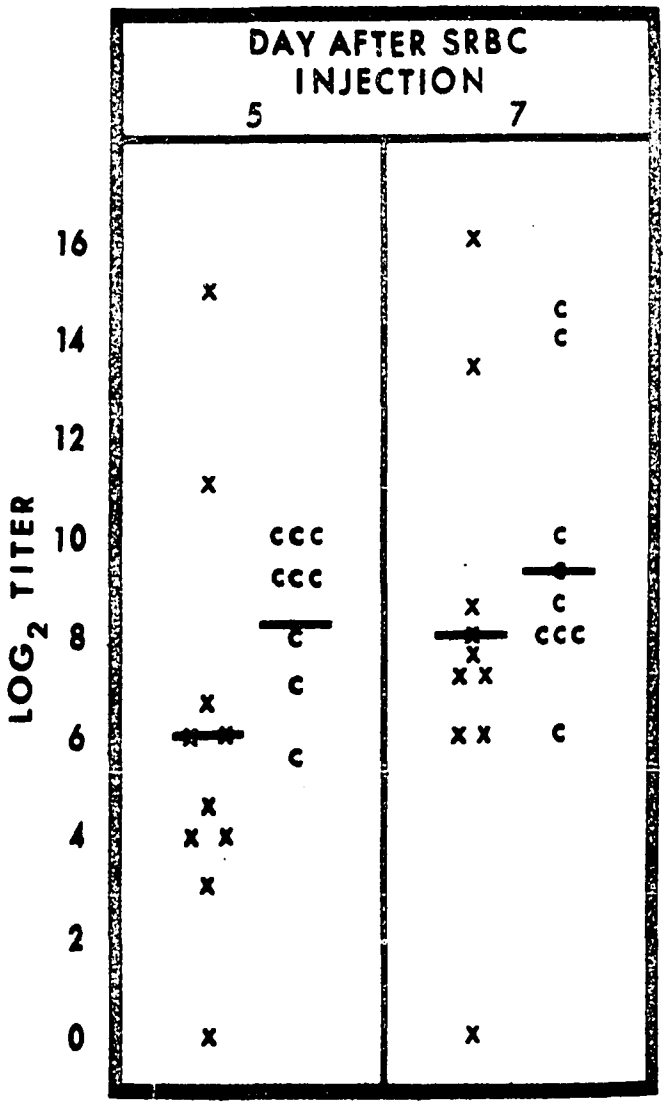


Fig. 20.--Individual antibody titers of control (c) and thyroidectomized (x) birds 5 and 7 days after sheep erythrocyte injection. The horizontal line in each group is the mean.

TABLE 16

MEAN TOTAL BLOOD LEUCOCYTE AND LYMPHOCYTE LEVELS OF 38-DAY-OLD CONTROL AND THYROIDECTOMIZED BIRDS

Treatment	No. of Birds	Total Leucocytes per mm^3[a]	% of Total Leucocytes[b]					Total Lymphocytes per mm^3[a]
			Lymph	Hetero	Mono	Baso	Eos	
Thyroidectomy	10	14,600±5,460[b]	67	24	6	2	1	10,200±4,500[c]
Control	9	21,200±4,900	78	15	5	2	0	16,400±3,800

[a] ± 1 standard deviation.

[b] Lymphocytes, heterophils, monocytes, basophils, eosinophils.

[c] p=.02 as compared to control values.

[d] p=.01 as compared to control values.

TABLE 17

SKIN GRAFT REJECTION TIME OF 56-DAY-OLD CONTROL AND THYROIDECTOMIZED BIRDS

Treatment	No. of Birds	Range of Rejection Time[a]	Mean Rejection Time[a]
Thyroidectomy	8	6-9	7.5
Control	5	7-8	7.2

[a]Time in days.

Anti-somatotropic hormone. Rabbit anti-bovine somatotropic hormone (ASTH) was injected into embryos to determine its effect on hatchability, body weight, and the immune response. Groups of embryos injected with normal rabbit serum (NRS) or saline were included as controls.

No difference was found in the hatchability of embryos in these groups. Mean body weights of the three groups were not significantly different throughout the experiment (Table 18). One bird, however, injected with ASTH had a body weight of about half of the mean weight of the other birds at all times during the experiment.

All birds were injected with sheep erythrocytes when they were 16 days old. Antibody responses of the ASTH treated birds were not significantly different from either of the other groups (Table 19). Histological examination of bursa, thymus, and spleen revealed no significant differences between ASTH and NRS treated birds.

A passive cutaneous anaphylaxis inhibition reaction was used to determine any reactivity that the anti-somatotropic hormone sera might have against antigens in chicken serum, chicken pituitary extract, or mouse serum, all presumably containing STH. Mouse serum was included as a positive control since it has been reported that it will inhibit a passive hemagglutination assay (121). Slight inhibition of the reaction was accomplished with mouse serum and possibly chicken serum (Table 20). The serum did not form

TABLE 18

MEAN BODY WEIGHTS OF BIRDS INJECTED WITH ANTI-SOMATOTROPIC HORMONE (ASTH) OR NORMAL RABBIT SERUM (NRS)

Treatment	No. of Animals	Age in Days		
		6	13	44
ASTH	6	31[a]	79	437
NRS	6	34	86	395
Saline	8	33	85	442

[a]Mean body weight in grams.

TABLE 19

ANTIBODY RESPONSE OF BIRDS INJECTED WITH ANTI-SOMATOTROPIC HORMONE (ASTH) OR NORMAL RABBIT SERUM (NRS)

Treatment	No. of Animals	Day after Antigen Injection		
		3	6	9
ASTH	6	3.5[a]	4.1	2.5
NRS	6	1.9	3.6	3.0
Saline	8	3.4	6.0	4.3

[a]Mean $\log_2$ anti-sheep erythrocyte titer.

TABLE 20

INHIBITION OF PASSIVE CUTANEOUS ANAPHYLAXIS REACTIVITY OF ANTI-BOVINE SOMATOTROPIC HORMONE WITH CHICKEN SERUM, CHICKEN PITUITARY EXTRACT, OR MOUSE SERUM

ASTH[a] Incubated with:	PCA Titer					
	10[b]	20	40	80	160	320
Saline	+++[c]	+++	+++	+++	++	+
Alkaline Saline	0	0	0	0	0	0
STH in Alkaline Saline	0	0	0	0	0	0
Young Chicken Serum	+++	+++	+++	+++	+	0
Adult Chicken Serum	+++	+++	+++	+	+	0
Chicken Pituitary Extract	+++	+++	+++	+++	+++	0
Mouse Serum	+++	+++	0	0	0	0

[a] Anti-bovine somatotropic hormone with a PCA titer of 320.

[b] Reciprocal of highest antiserum dilution causing reaction at skin test site.

[c] Relative degree of reaction at skin test site.

precipitin lines when tested by gel diffusion with chicken pituitary extract.

Anti-chicken pituitary extract. The failure of anti-bovine somatotropic hormone to alter the immune response could have been due to antigenic differences between chicken and bovine growth hormones. To circumvent this problem, rabbit antiserum to chicken pituitary extract was prepared. A globulin fraction of this serum was shown by double diffusion precipitation to contain a titer of 4 when assayed with undiluted chicken pituitary extract. Three precipitin lines formed. Two of these lines formed bands of identity with chicken serum, but not with rabbit serum. Groups of birds were injected with the gamma globulin fraction (ACPE), normal rabbit globulin (NRG), or saline. Injections of 0.2 ml ACPE containing 40 mg protein per milliliter and injections of 0.3 ml NRG containing 26 mg protein per milliliter were given on days 0, 3, 6, 9, 13, and 17 after hatching. Injections were intramuscular except for the last two, which were intravenous.

All birds were injected with 0.5 ml 10% sheep erythrocytes on day 13 after hatching. A second injection of 0.2 ml 10% sheep erythrocytes was given on day 20 after primary immunization. The antibody titer of birds injected with ACPE was significantly lower ($p=.05$) than that of the NRG injected birds on day 7 after primary antigen injection (Table 21). This did not represent a difference in peak

TABLE 21

ANTIBODY RESPONSE OF CHICKS INJECTED WITH ANTI-CHICKEN PITUITARY EXTRACT (ACPE) OR NORMAL RABBIT GLOBULIN (NRG)

Treatment	No. of Animals	Days after Antigen Injection[a]						
		4	7	13	20	23	25	29
ACPE	10	4.2[b]	3.5[c]	2.6	3.6	7.3	11.9	6.8
NRG	9	3.8	5.4	3.2	3.1	5.8	9.6	5.7
Saline	5	3.5	4.2	2.9	2.4	8.2	12.8	7.0

[a]A second injection of antigen was given 20 days after the first antigen injection.

[b]Mean $\log_2$ anti-sheep erythrocyte titer.

[c]$p=.05$ when compared with NRS injected birds.

titers, however, since the mean peak titer of ACPE treated birds occurred on day 4, which was 3 days earlier than in the NRG group. The injection of NRG did not alter the antibody response as compared to saline injected birds.

Absolute or relative bursa and spleen weights were not significantly different in ACPE or NRG treated birds at 38 days of age (Table 22). The mean total blood leucocyte count for ACPE treated birds was 15,500±1,800 (S.D.) per cubic millimeter on the 18th day of age. NRG injected birds had mean leucocyte count of 19,500±3,200 (S.D.) per cubic millimeter. These values were significantly different ($p=.01$) from each other.

Effect of Thyroxine and Somatotropic Hormone on Ontogeny of the Immune Response

Before studying the effect of hormones on immunological maturation, the time of normal appearance of the ability to produce antibody against sheep erythrocytes was studied. Groups of Hyline line 96 chicks were injected with 0.25 ml of a 10% sheep erythrocyte suspension divided equally into each leg on day 3, 4, 5, 6, 9, 12, and 15 after hatching. Antibody titers for each group were determined 7 days after the antigen injection. The first substantial antibody response in terms of both titer and the number of animals responding ($\log_2$ titer greater than 2) occurred on the 12th day after hatching (Table 23). Since this response appeared rather late in post-hatching development, the experiment was

TABLE 22

ORGAN WEIGHTS OF BIRDS INJECTED WITH ANTI-CHICKEN PITUITARY EXTRACT (ACPE) OR NORMAL RABBIT GLOBULIN (NRG)

Treatment	Body[a]	Bursa[b]	Bursa[c] / Body	Spleen[b]	Spleen[c] / Body
ACPE	364±28	2.11±.38	4.80±.74	0.85±.20	2.43±1.5
NRG	370±17	2.07±.50	5.64±1.5	0.82±.28	2.30±1.5

[a]Weight in grams ± 1 standard deviation.

[b]Weight in milligrams ± 1 standard deviation.

[c]Organ weight in milligrams divided by body weight in grams.

TABLE 23

ONTOGENY OF ANTIBODY FORMING CAPACITY
IN HYLINE LINE 96 CHICKENS

	Day Post-Hatching of Antigen Injection[a]						
	3	4	5	6	9	12	15
Mean $\log_2$ titer[b]	0.7	1.3	1.1	1.8	2.1	4.1	4.5
Number of responders[c]	0/10	2/10	1/10	2/10	3/9	6/8	10/10

[a]0.25 ml 10% sheep erythrocytes (0.125 ml into each leg).

[b]All titers determined 7 days after antigen injection.

[c]Log_2 titer greater than 2.0.

repeated using outbred White Leghorn chicks and two different antigen doses.

A 10% sheep erythrocyte suspension was injected in doses of either 0.05 ml/10 g body weight or 0.5 ml per bird to groups of birds on day 5, 8, 12, or 15 post-hatching. Antibody titers of each group were determined 7 days after antigen injection. The first substantial antibody response in birds receiving antigen amounts graded to body weight occurred on day 8 (Table 24). The mean amount of antigen injected on day 8 was 0.3 ml. Birds receiving 0.5 ml sheep erythrocytes responded by day 5 (Table 24).

Using these estimates for the time of appearance of antibody forming capacity, two experiments were performed to test the ability of thyroxine or STH to increase the maturation rate of the immune system. In the first of these experiments, chicks were injected daily from the day of hatching with 1) thyroxine (3 ug in 0.1 ml) 2) STH (1 mg in 0.1 ml), or 3) saline (0.1 ml). On the third day post-hatching, all birds were injected with 0.25 ml 10% sheep erythrocytes (0.125 ml per leg). A second injection of 0.25 ml 10% sheep erythrocytes was given 10 days after the first injection of antigen.

Chicks injected with saline produced essentially no antibody response to a first injection of antigen (Table 25). Thyroxine treated chicks produced a significant ($p=.05$) amount of antibody 6 days after the first injection of antigen. The

TABLE 24

ONTOGENY OF ANTIBODY FORMING CAPACITY IN OUTBRED WHITE LEGHORN CHICKENS

Dosage of Antigen[a]		Day Post-hatching of Antigen Injection			
		5	8	12	15
0.05 ml/ 10 g body wt.	Mean $\log_2$ titer	0.2	3.9	6.6	not done
	No. responders[b]/total	0/5	5/5	5/5	not done
0.5 ml/bird	Mean $\log_2$ titer	3.5	2.8	7.0	8.7
	No. responders/total	4/5	4/5	4/4	4/4

[a] 10% sheep erythrocyte suspension injected intramuscularly into right leg.

[b] Log_2 titer greater than 2.0.

TABLE 25

EFFECT OF THYROXINE AND SOMATOTROPIC HORMONE ON ANTIBODY FORMING CAPACITY AFTER INJECTION OF SHEEP ERYTHROCYTES ON DAY 3 POST-HATCHING

Treatment	No. of Animals	Day after Antigen Injection[a]					
		3	6	9	13	16	29
Thyroxine	4	1.4[b] 2[c]	2.5[d] 2	1.5 1	8.0[d] 4	8.8[d] 4	2.5 4
STH	5	1.0 0	1.5 1	2.1 2	6.6 5	8.6[d] 5	4.4[d] 5
Saline	4	0.8 0	0.3 0	0.5 0	6.3 4	6.9 4	2.6 4

[a]A second injection of antigen was given on day 10.

[b]Mean $\log_2$ anti-sheep erythrocyte titer.

[c]Number of responders ($\log_2$ titer greater than 2).

[d]$p=.05$ as compared to control values.

group injected with STH produced a slight response on day 9. A greater effect of the influence of thyroxine and STH on the immune response is seen in the response to the second injection of antigen. Both STH and thyroxine-treated chicks produced more antibody than did control birds. A significant difference was also seen in titers on day 29. This was 20 days after the second injection of antigen. The STH-treated group had a higher titer remaining than did the thyroxine or saline-treated groups. The larger drop in titer from day 16 to day 29 in the thyroxine-treated birds than in the normal control animals again suggests the increased catabolic rates present in thyroxine injected birds.

Another experiment was performed by treating outbred White Leghorn chicks from the day of hatching with: 1) daily 0.1 ml injections of thyroxine (5 ug), 2) daily 0.1 ml injections of STH (1 mg), 3) daily 0.1 ml injections of thyroxine (5 ug) and STH (1 mg), or 4) daily 0.1 ml injections of saline. On the 10th day post-hatching, 0.5 ml 10% sheep erythrocytes were injected into the right leg of each bird. A second injection of antigen was given 15 days later. Note that in the previous experiment, antigen was injected at 3 days of age.

Saline injected animals produced low levels of antibody after the first injection of antigen (Table 26). Slightly higher titers were seen in the hormone treated groups, but the only mean titer significantly different

TABLE 26

EFFECT OF THYROXINE AND SOMATOTROPIC HORMONE ON ANTIBODY FORMING CAPACITY AFTER INJECTION OF SHEEP ERYTHROCYTES ON DAY 10 POST-HATCHING

Treatment	No. of Animals	Day after Antigen Injection[a]						
		4	7	9	15	18	22	26
Thyroxine	7	4.1[b]	3.3	2.2	4.6	7.4	8.7	6.7
STH	9	5.4	5.2	3.1	5.9	8.2	8.1	6.3
Thyroxine and STH	8	5.8[c]	5.1	3.1	5.3	7.2	6.7	6.0
Saline	9	3.9	3.6	2.0	5.1	6.7	8.0	5.4

[a]A second injection of sheep erythrocytes was injected on day 15.

[b]Mean $\log_2$ anti-sheep erythrocyte titer.

[c]$p=.05$ as compared to saline treated group.

($p=.05$) from the normal controls was on day 4 after the first injection of antigen in the group treated with both thyroxine and STH. A point of interest in this experiment was the surprising increase in antibody titer which occurred between days 9 and 15. This increase was observed in all four groups. A normal secondary response occurred in all four groups.

CHAPTER IV

DISCUSSION

This research was directed toward defining the influence of developmental hormones in the ontogeny and maintenance of function of the avian lymphoid system. Of prime importance toward solution of this problem is the ultimate determination of the precise morphological and biochemical sites of action of the hormones. Toward this end, several areas of influence of thyroxine, STH, or both were indicated.

Developmental hormones may play a role in the normal development of the epithelial anlage of central lymphoid organs which forms a specialized microenvironment that can influence differentiation of stem cells into immunocompetent cells. Another possible role for developmental hormones could be the preparation or differentiation of stem cells to a stage that would be susceptible to influence by the central lymphoid organ microenvironment. Developmental hormones could also act on differentiating post-central lymphoid organ cells by preparing for, or aiding in the influences of central lymphoid organ humoral factors. These hypothetical sites of action suggest a role of hormones in differentiation

and maturation of precompetent lymphoid cells. It is also possible that the hormones cause expansion or proliferation of immunocompetent cells without effecting differentiation specifically.

Hormones may also influence the immune response through such other non-specific mechanisms as stimulation of phagocytosis or inflammatory processes. They may alter normal metabolic processes which regulate protein catabolism or synthesis essential to immune tissue function.

The present experiments characterizing the effects of developmental hormones on the immune system provide a basis for postulating sites and mechanisms of action of the hormones. The first group of experiments involved study of the effect of thyroxine on testosterone-treated embryos. Groups of animals treated as embryos with thyroxine, after testosterone treatment, produced more antibody upon challenge with sheep cells at 5 weeks of age than did birds treated as embryos with testosterone alone (Figs. 5 and 7). Similarities of antibody response in the various groups were noted in the first two experiments. Testosterone-treated birds produced very little antibody after the first injection of antigen. After a second antigen injection some birds responded, while others did not. In both experiments, groups treated with both testosterone and thyroxine contained more birds producing larger amounts of antibody after the second injection of antigen than groups of birds treated with testosterone alone.

Analysis of the serum of normal, testosterone, and testosterone and thyroxine-treated animals using density gradient ultracentrifugation revealed relatively less low molecular weight, nonagglutinating antibody in the serum of responders in both hormone-treated groups. Those animals treated with testosterone and thyroxine which responded to sheep erythrocytes made antibody qualitatively similar to that produced by responders treated with testosterone alone. It would appear, then, that thyroxine reversed the effect of testosterone which results in nonresponsiveness. On the other hand, thyroxine did not restore the animals to a normal state and, paradoxically, the elevated antibody titers in these animals were related to immune deficiency rather than hypercompetence.

The differences in quantity of antibody produced in the two experiments may be related to differences in quantity and method of antigen injection, i.e., in one experiment complete Freund's adjuvant was used. Differences may also be related to the dosage and timing of testosterone and thyroxine treatment.

Jankovic (80, 81) described adequate antibody responses after multiple injections of antigen in birds treated as embryos with testosterone. More complete bursectomy, accomplished either by surgery and X-irradiation (29), or by using larger amounts of testosterone (161), reduced the chance for a few cells to be influenced by the bursa

environment and reach the peripheral tissues. The fact that normal amounts of antibody could be produced by animals with severely limited central lymphoid organ function illustrates a relative degree of peripheral lymphoid system autonomy.

In experiments using testosterone-treated chicks, a few bursa-dependent cells escape hormonal bursectomy and reach peripheral lymphoid tissue. They proliferate upon antigen exposure and produce antibody. It must be assumed that when a larger number of cells escape, a greater amount of antibody will be produced at a given time after antigen injection. Embryonic thyroxine injections after testosterone treatment produce a larger number of animals that can respond by antibody formation after a second antigen exposure. The mechanism of this effect is not clear. Thyroxine may antagonize the detrimental effect of testosterone in one or more ways. In any case, a larger number of bursa-dependent cells would be expected to reach peripheral lymphoid tissues and subsequently produce more antibody.

In order to speculate on the mechanism of this thyroxine effect, the postulated mechanisms of testosterone bursectomy must be considered. Testosterone has been suggested to effect hormonal bursectomy by several mechanisms. It may prevent the entry or binding of stem cells in the bursa (106). Testosterone may also have a direct antimitotic activity on bursa cells (129). Ackerman and Knouff (1) have shown that testosterone prevents alkaline

phosphatase activities which may be necessary for development and function of the epithelial anlage of the bursa. Thyroxine increases mitotic activities in lymphoid organs (43) and also increases tissue and serum levels of alkaline phosphatase (89, 153).

In mice, relationships between the thymus and pituitary have been found (119). Neonatal thymectomy causes changes in the acidophilic STH producing granules in the hypophysis. Removal of the thymus also causes changes in the thyroid gland (71, 72). Two weeks after thymectomy, thyroid weight, acinar cell height, and oxygen consumption were increased. These parameters had returned to normal values at 4 weeks after thymectomy. Observations similar to these have been made in the chicken by Pintea and Pethes (125). One month after surgical bursectomy, thyroid follicles were increased in size and I^{131} uptake was decreased, indicating a decreased thyroid function. At 74 days of age, follicle size was significantly smaller than in normal birds.

In the present studies, a change in thyroid morphology was observed after testosterone treatment on the 12th embryonic day. Follicle diameter was decreased as compared to the diameter of thyroid follicles in normal 18 day embryos (Table 1). This may represent a compensatory increase in thyroid hormone output related to bursa destruction. On the other hand, the change in thyroid morphology might be a result of direct testosterone action on the thyroid. These

observations are offered only as an indication of the interrelationship between lymphoid organs and the thyroid gland.

Thyroxine may also influence proliferation of bursa-dependent cells in peripheral lymphoid tissue. This does not seem likely in this experimental design since thyroxine was injected in small quantities into embryos. The effect of exogenously administered thyroxine is short-lived (152), and injected thyroxine would not have been detectable five weeks later at the time of antigen injection. A short period of exposure of bursa-dependent cells to thyroxine, however, may be adequate to expand the population to a crucial level. These cells could be expected to continue normal expansion and produce detectable amounts of antibody five weeks later.

The histological appearance of the bursa of testosterone and thyroxine-treated embryos was not strikingly different from that of embryos treated with testosterone only. Lymphoid development was severely reduced in both groups. This evidence indicates that the probable site of action of thyroxine was outside the bursa. It is possible, however, that thyroxine caused important changes in the developing bursal environment which were not detected by histological methods on the 18th embryonic day.

High levels of thyroxine cause lymphoid tissue atrophy, as compared to stimulating influences of lower amounts of the hormone (3, 4). These observations were confirmed at a functional level (Table 4). Injection of 0.5 ug thyroxine

on embryonic day 12 and 2 ug on embryonic day 15 resulted in reduced antibody forming capacity of birds challenged with sheep erythrocytes at 5 weeks of age. This effect was present during the primary response but was not seen after a second injection of antigen. This indicates that the number of competent bursa-dependent cells was lowered by excessive embryonic thyroxine treatment. The cells that escaped the effect were able to expand their population and produce increased amounts of antibody on second exposure to antigen. This dose of thyroxine may act directly on the bursal environment or may increase adrenal cortical steroid production which has been shown to produce bursal atrophy (62).

The effect of STH on testosterone-treated birds was also studied. Daily STH injection of birds treated as embryos with testosterone did not increase antibody production above values for birds treated with testosterone alone (Table 5). The dose of testosterone in this experiment was larger than that used in other experiments. This may have limited the small population of cells in the peripheral tissues that would be available for population expansion by STH. Since STH was injected after hatching, alteration of the bursa microenvironment during development was not likely.

Although some alteration in lymphoid organ weights can be produced by injections of STH and thyroxine in chickens (12, 63, 157) little study has centered around the effect of these hormones on the functional immune response. In

experiments with chicks and adult birds, little influence of thyroxine or STH injections was seen on antibody formation or skin graft rejection (Tables 6-8).

These results must be considered with reference to the dosages of the hormones used. Adult birds, 6 weeks old, were injected with 18 ug thyroxine per day. This dosage was increased after 16 days to 30 ug per day. The 30 ug dosage was approximately 10 times the daily secretion rate of thyroxine in normal 7-week-old birds (76, 166). Four milligrams of STH were injected daily. This dose was equivalent to that used by Glick (63) to increase the weight of the bursa of Fabricius. The dosages, therefore, should have been large enough to cause increased levels of hormones in the birds. Normal body compensatory mechanisms, however, must be considered. In guinea pigs, a compensatory effect of the thyroid and adrenal glands has been reported (94). In chicks, metabolic rates which were increased by feeding thyroprotein fell below normal values several hours after feeding was discontinued (99). The birds were actually hypothyroid at the time of the metabolic rate determinations since continuous thyroprotein feeding had depressed thyroid size and function and after stopping treatment, birds received no exogenous thyroprotein.

Essentially no difference was seen between groups that received hormones throughout the experiment and groups in which treatment had been discontinued. Therefore, a

hypothyroid condition after stopping thyroxine treatment could not be shown to alter antibody formation.

The effect of decreased thyroxine levels produced by thiouracil treatment was studied in birds of various ages. Since thyroxine injections were able to influence antibody response of birds injected previously with testosterone, thyroxine might be essential to normal development of the bursal environment. Thiouracil was injected into embryos on the 5th or 12th embryonic day in an attempt to reduce their thyroxine levels. Thyroxine is first produced in the embryonic thyroid around the 10th day of incubation (143, 156). Enlarged thyroids and delayed hatching and yolk sac retraction time have been described as symptoms of an embryonic hypothyroid condition (2, 134). Because thiouracil was injected as early as the 5th embryonic day and these symptoms were observed, a hypothyroid state was probably attained.

This condition did not alter the lymphoid nature of the bursa on the 18th embryonic day. Antibody formation and skin graft rejection time were not suppressed when animals were tested at 5 weeks of age (Tables 9 and 10). These experiments indicate that thyroxine is not solely responsible for supporting a critical stage in the development of the bursa microenvironment. If so, then thiouracil did not sufficiently block thyroxine production. Another complication would be the presence of maternal thyroxine in the eggs.

To test the role of thyroxine in supporting normal

lymphoid tissue functions thiouracil was injected into newly hatched chicks and adult birds. Although the amount of thiouracil injected daily (1 to 10 mg) was comparable to that (0.1% in feed) shown by several investigators to have a maximum anti-thyroid effect (99), normal morphology of the thyroid glands at the end of the experiment indicated that a hypothyroid condition was not attained. Some information, however, can be gained from the antibody response and skin graft rejection data in these experiments (Tables 11-14). If a hypothyroid condition was not attained, then the effect of additional hormone injections can be studied in some groups of birds in these experiments. Birds, both chicks and adults, treated with thyroxine and STH after discontinuing thiouracil treatment failed to demonstrate any increase in antibody titer above that found in saline-treated control birds (Tables 11 and 12). This confirms the results seen in previous experiments with different hormone dosages (Tables 6 and 8).

Because attempts at producing a hypothyroid condition by injecting thiouracil were unsuccessful, birds were treated with thiouracil in the feed (0.1%). At 37 days of age, relative (organ weight/body weight) spleen, and bursa weights of birds fed thiouracil were only 80% of those of normal birds. At 80 days of age, relative spleen and bursa weights were 93 and 95%, respectively, of control values. This increase in relative organ weight of thiouracil treated birds from 37 to

80 days of age could be a reflection of a less severe hypothyroid condition at 80 days of age, as seen by smaller goitrogenic thyroids at 80 days than at 37 days. These results confirm the decrease in relative spleen and bursa weights observed by Garren and Shaffner (60) with thiouracil treatment in young birds.

The effect of thiouracil-induced hypothyroidism on lymphoid organ weights is more easily shown than an effect on the immune response. Antibody formation and skin graft rejection times of thiouracil-treated birds did not differ from normal birds (Fig. 9).

In contrast to earlier experiments in which thiouracil was injected, thiouracil feedings produced an enlargement of the thyroid glands. This goitrogenic effect is indicative of some level of hypothyroid condition (133).

In order to attain a more complete hypothyroid state, surgical thyroidectomy was performed. At 95 days of age, the relative spleen weight of thyroidectomized birds was only 78% of that of normal birds. The finding of normal bursa weights in thyroidectomized birds might be explained by the observations of Glick (61) on the time of regression of bursas of normal birds. In White Leghorn chickens the bursa becomes smaller in relation to body weight between 4 and 7 weeks of age. At 95 days of age in this experiment, bursas of normal birds could have begun regression. This normal regression of the bursa is dependent on function of the gonads

(61). Because thyroidectomy lowered not only protein catabolism and lymphoid tissue cellularity, but also normal development of the testis (17), bursas in thyroidectomized birds would not be subject to normally occurring age involution. This could allow bursa weights to appear as large in thyroidectomized birds as in normal birds at 95 days of age. It was shown previously that thiouracil treated birds had significantly lower relative bursa weights than did control birds.

Peak antibody titers to sheep erythrocytes and Salmonella organisms were lower in thyroidectomized birds than in normal birds (Fig. 16). This agrees with the results seen in thyroidectomized guinea pigs injected with Salmonella organisms (112) or egg albumen (111). Lower agglutinating and precipitating antibody responses were observed in thyroidectomized guinea pigs as compared to controls. The slower rate of decline in antibody titer noticed in thyroidectomized chickens, compared to normal birds, agrees with observation that thyroidectomy of rats leads to decreased catabolism of serum proteins (56). This may also explain why no correlation exists between visible symptoms of thyroidectomy (size of bird and appearance of feathers) and levels of antibody (Fig. 17). In these most severely hypothyroid birds, the rate of catabolism of serum proteins may be reduced to such an extent that a small rate of antibody production would appear as a high titer of antibody.

Histological examination of the thymus at 95 days of age revealed fewer lymphocytes in the cortical areas of thyroidectomized birds than in normal birds (Figs. 18 and 19). This is in agreement with the effects of thyroidectomy on lymphoid organs in several species of animals (cf. 34).

A second experiment was performed in order to study the effect of thyroidectomy on skin graft rejection. The growth rate and appearance of thyroidectomized birds in the second experiment were comparable to those in the first experiment. Sheep erythrocytes were injected into birds at 20 days of age. In contrast to the first experiment, in which 0.5 ml of a 10% sheep erythrocyte suspension was injected into each bird, sheep erythrocytes were injected on a body weight basis. The mean amount injected into controls was 0.74 ml compared to 0.57 ml for the thyroidectomized birds.

Mean antibody titers in this experiment were not significantly different for normal and thyroidectomized birds (Table 15). The individual titers revealed that two thyroidectomized birds had very high titers (Fig. 20). As in the previous experiment, some of the smallest birds produced the highest titers. If thyroidectomy, as a whole, was more complete in this experiment, then the catabolism of serum proteins could have been sufficiently reduced to mask any differences in antibody titers in the two groups of birds. This, however, is not likely because an increased amount of

antibody was not seen in the thyroidectomized birds near the end of the bleeding schedule. A more likely explanation would be that the slightly larger amount of injected antigen stimulated more cells in the thyroidectomized birds to respond, and by the day of the peak antibody titer any differences in normal and thyroidectomized birds would not be seen.

At 38 days of age, total leucocyte and lymphocyte levels were reduced in thyroidectomized birds (Table 16). Although reports in the literature concerning changes in lymphoid organ weights after thyroidectomy or thyroxine injection are abundant, peripheral blood changes have been less well documented (34). The reduced lymphocyte levels may indicate a thymus-dependent cell deficiency. This, however, was not demonstrable by skin graft rejection times (Table 17). In guinea pigs, skin graft rejection times were not altered after thyroidectomy even though deficiencies in response to DNCB were found (113 and 114).

The studies in which hypothyroid conditions were produced by thiouracil or thyroidectomy indicate that thyroxine has a role in maintaining central and peripheral organ weight, antibody production, and peripheral leucocyte levels. Low levels of thyroxine during embryonic development do not have a permanent effect on the bursa environment that is demonstrable by a reduction in antibody production. Skin graft rejection did not appear to be as sensitive to thyroidectomy as peripheral blood lymphocyte levels.

In order to study the effect of reduced somatotropic hormone levels, rabbit anti-bovine somatotropic hormone (ASTH) was injected into embryos. The hatchability of these birds was not affected, as compared to control birds injected with normal rabbit serum (NRS). Repeated injections of birds with ASTH or NRS did not alter body weight or antibody formation (Tables 18 and 19).

The passive cutaneous anaphylaxis inhibition test revealed that ASTH had little reactivity against chicken serum or chicken pituitary extract (Table 20). ASTH did not form precipitin lines when tested by gel diffusion with chicken pituitary extract. These results would explain the lack of ability of STH to increase body weight in chickens (93). Glick (63), however, reported some influence of STH on bursa weight.

In order to prepare an antiserum that might effectively lower the level of STH in chickens, chicken pituitary extract was injected into rabbits. A globulin fraction of the rabbit serum was then prepared. This anti-chicken pituitary extract (ACPE) was injected into newly hatched birds. Such birds demonstrated a significantly lower antibody level to sheep erythrocytes, as compared with control animals, at only one time interval during the immune response (Table 21). This did not represent a difference in peak antibody titers since the peak titer for ACPE treated birds occurred 3 days earlier than in birds treated with NRG. Since determinations

were not made between days 4 and 7, the exact time of occurrence of peak antibody titer could not be determined for each group. However, a difference in the time of appearance of antibody is suggested.

The effect of ACPE on the immune response in chickens was not as severe as that reported following injection of anti-mouse pituitary serum into mice (120 and 123). Anti-pituitary serum caused severe lymphocyte depletion in thymus and spleen, as well as a wasting syndrome in some mice.

On day 18 after hatching, a reduced number of total leucocytes was seen in birds treated with ACPE. However, no organ weight differences between groups of birds were observed when the birds were 38 days old (Table 22).

Only slight evidence of an effect of ACPE on the immune system in chickens was seen. One reason for this might have been the low titer of antibody present in the ACPE serum. Several injections of the antiserum were given in an attempt to overcome this problem. In contrast, mice given only one injection of anti-mouse pituitary serum showed signs of wasting (106).

In mice, treatment of neonatal thymocytes with STH will cause a maturation which allows these cells to function in a graft versus host assay system (118). Experiments were performed to test the possibility that injections of STH or thyroxine might increase the rate of maturation of the capacity of newly hatched chicks to produce antibody. The time

of appearance of antibody forming capacity was dependent on the amount of a 10% sheep erythrocyte suspension used as the challenging antigen. The first substantial antibody response to an injection of 0.25 ml of the suspension appeared on day 12 after hatching (Table 23). An antigen dose of 0.05 ml/10 g body weight (approximately 0.3 ml on day 8) caused a response in 8-day-old birds (Table 24). Injection of 0.5 ml of antigen caused an antibody response in 4 out of 5 birds when injected on the 5th day after hatching. Genetic differences in some of the experimental animals may also be responsible for the timing differences. Birds injected with 0.25 ml antigen were line 96, while the other groups were outbred White Leghorn chicks. Antibody responses of these birds were determined on day 7 after antigen injection. Later experiments (Table 26) indicated that in these young chicks the peak titer occurred before day 7. Thus, the ability of normal chicks to respond to sheep erythrocytes probably is present to some degree before the stated times.

In line 96 chicks injected with 0.25 ml 10% sheep erythrocyte suspension on the 3rd day post-hatching, antibody response was greater in chicks injected with thyroxine than in normal birds (Table 25). A second injection of antigen (0.25 ml) was given on embryonic day 13. Normal chicks produced antibody after this challenge. Chicks injected with thyroxine or STH, however, produced significantly larger amounts of antibody than did normal chicks. On day 29 after

the first injection of antigen, birds injected with STH retained a higher amount of antibody compared to thyroxine-treated and normal birds. The greater loss of titer in birds injected with thyroxine reflects, as in previous experiments, the role of thyroxine in altering rates of catabolism of serum proteins.

In another similar experiment, outbred White Leghorn chicks were injected with sheep erythrocytes (0.5 ml) on the 10th day posthatching. In this experiment, all groups of birds produced antibody after the first injection of antigen. On day 4 after antigen injection, chicks injected with thyroxine and STH had significantly higher antibody titers than did the other groups. All birds showed increases in antibody titer from the 9th to 15th days after the first injection of antigen. These results are unexplained, but since antibody also increased in normal birds, they were not due to hormone treatments.

On the basis of these experiments, it can be concluded that STH and thyroxine increase the immune response in birds around the time they are developing the capacity to respond. This is not necessarily proof of an increase in the maturation rate of antibody formation, since the largest increase in antibody formation was seen in secondary responses.

A thymus-produced hormone has been well established as a factor which supports maturation of thymus-dependent lymphocytes in peripheral tissues (154 and 163). Proof of

the existence of a hormone produced by the bursa is less readily available. Supporting evidence has been presented (82, 83, 135). However, the effects have also been attributed to the endotoxin theoretically present in any conventional bursa preparation (31).

The developmental hormones exert many of the actions attributed to hormones produced by central lymphoid organs. Developmental hormones may aid, or be necessary for the action of central lymphoid organ hormones.

Since removal of central lymphoid organs may alter thyroid or pituitary function (71, 72, 118, 119), the central lymphoid organ hormone may be postulated to regulate, to some extent, the role of developmental hormones in maintaining the population of immunologically competent cells in the peripheral tissue.

In the present investigations, the influence of developmental hormones on immune lymphoid tissue function was studied with reference to three specific aspects of the immune system. These were: 1) essential events occurring in the central lymphoid organ microenvironment, 2) differentiation of the immune system to competency in the newly hatched period, and 3) support of lymphoid tissue responses around the time of exposure to antigen.

In the chicken, developmental hormones exert influence to some extent in all of these stages. Analysis of all data presented suggests that the dependence of lymphoid

tissues on developmental hormones in the chicken is not qualitatively different from that found in mammals. The clear demonstration of cause and effect relationships at every level has not yet been possible. It is felt that this was due to the following complications: 1) difficulty in obtaining and maintaining sufficiently inbred chickens to reduce normal variance to a desirable level, 2) lack of availability of specific avian hormones such as somatotropic hormone, thyrotropic hormone, and others, 3) inability to provide ideally controlled environments for the support of immunologically deficient experimental animals, and 4) difficulty in maintaining constant environmental conditions to minimize the effects of endogenous hormonal variation. More strict control of these variables may lead to greater evidence of the interactions of developmental hormones with lymphoid tissue.

CHAPTER V

SUMMARY

The influence of somatotropic hormone and thyroxine on the immune system of chickens was studied with reference to three periods in immunological development. Central lymphoid organ development, maturation of antibody forming capacity, and support of the immune response at the time of antigenic exposure were studied.

Injections of thyroxine into embryos can allow bursa-dependent cells to become competent after testosterone treatment which normally blocks central lymphoid organ function of the bursa and leads to deficiencies in the bursa-dependent population of cells. Evidence for a relationship between the bursa and thyroid was observed. Changes in thyroid morphology were seen after injection of testosterone.

Hypothyroid conditions induced by thiouracil or surgical thyroidectomy resulted in lower body, spleen, and bursa weights. Antibody responses to sheep erythrocytes and Salmonella organisms were lower than normal in thyroidectomized birds. Total peripheral blood leucocyte and lymphocyte levels were lowered by thyroidectomy, however, skin

graft rejection time was not altered.

Injection of anti-chicken pituitary extract into birds had slight influence on antibody production but did reduce peripheral blood leucocyte levels. Body, spleen, and bursa weights were not altered. Anti-bovine somatotropic hormone caused no alteration of body weight or antibody formation. Little reactivity was seen between anti-bovine somatotropic hormone serum and chicken pituitary extract or chicken serum, using gel diffusion and passive cutaneous anaphylaxis reactions.

Somatotropic hormone and thyroxine caused a slight increase in the rate of maturation of antibody forming capacity. Chicks injected daily with both hormones developed capacity to produce antibody at an earlier age than did normal birds.

These results suggest that developmental hormones may not be the most critical factor in development of immune capacity, however, it is strongly indicated that hormones do influence the expression of that capacity. The dependence of lymphoid tissues on developmental hormones in chickens does not appear qualitatively different from that found in mammals.

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