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Effects of Triiodothyronine Treatments on Body and Organ Growth and the Development of Immune Function in Dwarf Chickens[1] (41915)

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Abstract. The effects of triiodothyronine (T_3) treatments on general body growth, long bone growth, primary lymphoid organ development, antibody production, and serum growth hormone (GH) and thyroid hormone levels were examined in two dwarf strains (sex-linked dwarf—SLD, and autosomal dwarf—ADW) and in a normal-growing control strain (K) of White Leghorn chickens. One-day-old male chicks from each of these strains were assigned to either an untreated control group or to one of the groups receiving a T_3 supplement ranging from 0.01 to 1.0 ppm. General body growth and long bone growth were significantly ($P < 0.05$) stimulated only within the SLD strain by the intermediate T_3 dosages. The 1.0-ppm T_3 dosage level resulted in depressed body weights within both the K and ADW strains but produced no significant changes within the SLD strain. Thymic growth was significantly stimulated due to treatments of 0.1 ppm T_3 in the SLD strain ($P < 0.05$) and 1.0 ppm T_3 in both the SLD and ADW strains ($P < 0.001$ and $P < 0.05$, respectively). Bursal growth was significantly depressed ($P < 0.05$) at all T_3 dosage levels within the SLD strain while 0.01 and 0.1-ppm T_3 treatments resulted in significant bursal growth stimulation in the K and ADW strains, respectively. Concomitant with the depressed bursal growth, antibody production was significantly depressed ($P < 0.05$) within the SLD strain at the 1.0-ppm T_3 dosage level. Antibody production was not significantly affected by any of the T_3 treatments within the control K or ADW strains. Serum T_3 levels were significantly increased in all strains by the T_3 supplementation but thyroxine (T_4) serum levels were affected only within the SLD strain. The 0.01-ppm T_3 treatment resulted in a significant increase ($P < 0.05$) in serum T_4 levels within this strain and treatment group. The only increase ($P < 0.05$) in GH levels due to T_3 treatments occurred within the same SLD treatment group. The higher T_3 treatments resulted in serum GH levels being severely depressed ($P < 0.01$) in all strains.

The hormonal milieu of the animal exerts important controls on growth and development. The role of neuroendocrine function in immune system development and function is an area that has only recently begun to generate considerable research interest. Of the investigations thus far, many have used either one of the dwarf strains of mice (1–3) or *in vitro* approaches (4) for the study of these interactions.

The studies presented here have utilized two dwarf strains of chickens as models for investigating possible interactions between the immune and endocrine systems. One of these strains, the sex-linked dwarf (SLD), is known to have low peripheral 5′-monodeiodinase activity resulting in the impaired conversion of thyroxine to the more biologically active triiodothyronine (5), abnormally fluctuating growth hormone levels (5, 6), and low somatomedin levels (7). These hormonal deficiencies are accompanied by impaired humoral immune function (8) which can be modified by hormonal manipulations (9). The second dwarf strain, the autosomal dwarf (ADW), has been found to also have abnormally fluctuating growth hormone levels and slightly depressed thyroid function (5). The growth of this strain is greatly reduced relative to the control strains but the immune parameters examined thus far appear to be normal (8, 9).

The purpose of these experiments was to further examine the effects of hormonal treatments in these strains and to gain further information on the roles of growth hormone and the thyroid hormones in regulating body growth, specific organ and tissue growth, and immune function.

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Materials and Methods. *Experimental animals.* Three strains of White Leghorn chickens developed and maintained at Cornell University were used in these experiments. These were the sex-linked dwarf (SLD) strain (10), the autosomal dwarf (ADW) strain (11), and a normal-growing control (K) strain. The autosomal dwarfing mutation first arose within the K strain and both strains express only the B^{15} major histocompatibility complex (MHC) haplotype. The sex-linked dwarfing gene (*dw*) was incorporated into the same White Leghorn population from which the K strain was originally derived and has been maintained as a separate strain for many years. Both the B^{15} and B^{19} MHC haplotypes are found within this strain. Chicks from each strain were randomly assigned to treatment groups and housed in thermostatically controlled battery brooders with raised wire floors. A commercial chick starter mash (Agway Pacemaker) and water were provided *ad libitum*. The chicks were raised on a 15L:9D lighting regime.

Experimental protocol. Experiments 1 and 2 were performed by two separate laboratories. Experiment 1 was designed to assess the effect of triiodothyronine (T_3) supplementation on general body growth in the dwarf strains. The second experiment examined the dose–response effects of T_3 supplements on immune development and function.

Experiment 1. At 2 weeks of age, male and female chicks from each of the three strains were randomly assigned to four treatment groups. The number of animals per treatment group is given in the results. The treatments for each of these groups were as follows: Group 1 served as the untreated control and was fed the unsupplemented commercial chick starter ration throughout the experiment; Group 2 was fed the commercial diet supplemented with 0.1 ppm T_3; the Group 3 diet was supplemented with 0.3 ppm T_3; and the Group 4 diet was supplemented with 1.0 ppm T_3. The treatments were continued over a 4-week period. Initial body weight measurements were taken at the

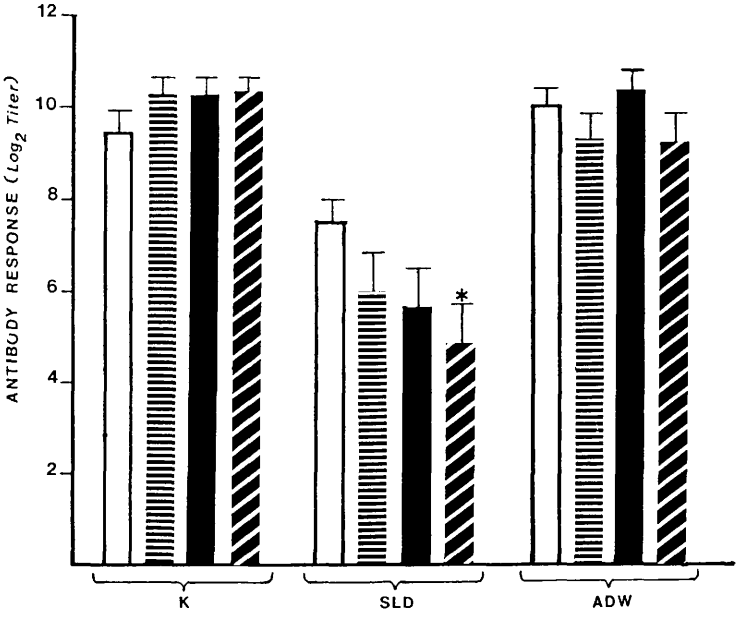

FIG. 1. The antibody response of the control K strain and the autosomal and sex-linked dwarf strains to SRBC. Each bar represents the mean circulating antibody titer for 15–18 male chicks per group with the SEM indicated. Open bars represent the control treatment group; horizontal slashed bars represent the 0.01-ppm T_3 treatment group; the solid bars represent the 0.10-ppm T_3 treatment group; and the diagonally slashed bars represent the 1.0-ppm T_3 treatment group. Significantly different from the control treatment within that strain, $*P < 0.05$.

beginning of the treatment period and again at the termination of the experiment. Also, at this time, measurements of shank length were taken for all animals and SLD pectoralis muscle and liver were collected and weighed.

Experiment 2. One-day-old male chicks from each of the three strains were randomly assigned to one of four treatment groups with 15–18 chicks per group. Group 1 was the untreated control group and was fed the unsupplemented commercial chick starter diet. Groups 2, 3, and 4 were fed the chick starter diet supplemented with 0.01, 0.1, and 1.0 ppm T_3, respectively. Body weights were recorded upon assignment to treatment groups and at weekly intervals thereafter. At 28 days of age, all chicks received a primary immunizing dose of suspended SRBC. One week later, blood samples were collected and the serum analyzed for antibody and hormone concentrations. At 36 days of age, final body weights were recorded and the animals sacrificed. All thymic tissue and the bursa of Fabricius were removed and cleaned of adherent tissue prior to obtaining organ weights.

Immunization and antibody titration. A 0.5% suspension of sheep erythrocytes (SRBC) in 0.01 *M* phosphate buffer, pH 7.4, containing 0.85% saline served as the test antigen. All chicks were immunized at 4 weeks of age by a 0.2-ml intracardial primary injection of the SRBC suspension. Blood samples were drawn by cardiac puncture at 5 weeks. All sera were individually analyzed for circulating anti-SRBC antibody and for hormone concentrations. Antibody was measured by the microhemagglutination technique (12) as described previously (8).

Triiodothyronine treatments and hormone determinations. T_3 supplementation was accomplished by slowly adding 10 ml of a 10 mg/ml solution of 3,3′, 5-triiodo-L-thyronine (Sigma Chemical Co.) per 10 kg of the commercial diet while mixing. After thorough mixing, this concentrate (10 ppm T_3) was used to prepare the 1.0 ppm T_3 supplemented diet. The 0.1 and 0.01 ppm T_3 supplemented diets were prepared using the 1.0 ppm T_3 stock diet.

The serum concentrations of thyroxine (T_4) and T_3 were measured in duplicate samples using commercially available Micromedic radioimmunoassay (RIA) kits. The sensitivities of the RIAs for the thyroid hormones were 4.3 ng T_4/ml and 0.16 ng T_3/ml. Avian growth hormone (GH) serum concentrations were assayed in duplicate at two concentrations in a single RIA using the specific homologous RIA developed by Harvey and Scanes (13).

Statistical treatment of data. All samples were coded and randomized for antibody and hormone determinations. The data were organized into the respective strain and treatment groups only after the samples had been read and recorded. Analysis of variance was performed to determine if strain or treatment effects existed within the different parameters examined. Planned comparisons were analyzed using the protected LSD *T* test (14).

Results. The body weight gain and shank length data from Experiment 1 are presented in Table I. Significant increases in body weight gain over the 4-week treatment period occurred only within the SLD strain chicks (both males and females) that received the lower (0.1 and 0.3 ppm) T_3 supplementation levels. The 1.0 ppm T_3 dosage did not significantly increase body weight gains in the SLD strain but did significantly depress this growth parameter in both the K ($P < 0.005$) and ADW ($P < 0.05$) strains.

The same treatments that had significant enhancing effects on SLD body weights were also stimulatory to long bone growth. Significant increases in shank length measurements were found in all female SLD treatment groups when compared to the untreated control. The same result was observed in the male SLD treatment groups, although enhancement of long bone growth was generally somewhat less than that observed in the females. Within the males only the 0.1- and 0.3-ppm T_3 treatments significantly increased ($P < 0.05$) shank length.

The effect of T_3 treatments on SLD liver and pectoralis muscle was monitored to provide additional growth indicators within this strain (Table II). When the liver weights were adjusted for body weight, the growth of this organ was depressed to a highly significant ($P < 0.005$) degree in all T_3 treatment groups for both males and females. This was a function of the increased body weights within these treatment groups, however, since there were no significant differences ($P > 0.05$)

TABLE I. EFFECT OF T_3 TREATMENTS ON BODY WEIGHT GAIN AND LONG BONE GROWTH

Treatment	K strain			SLD			ADW		
	Body wt gain (g)		Shank l. (mm)	Body wt gain (g)		Shank l. (mm)	Body wt gain (g)		Shank l. (mm)
Males									
Control	235.5 ± 19.7[a]	(13)	82.5 ± 2.1	138.4 ± 5.9	(11)	70.4 ± 1.6	200.7 ± 6.8	(8)	67.1 ± 0.9
0.1 ppm T_3	214.8 ± 15.1	(12)	84.92 ± 2.1	163.6 ± 14.4*	(11)	74.4 ± 1.0*	186.4 ± 13.2	(17)	69.6 ± 1.0
0.3 ppm T_3	197.8 ± 14.1	(13)	82.4 ± 1.6	186.9 ± 12.2***	(13)	75.2 ± 1.2*	191.1 ± 18.11	(8)	71.1 ± 2.0
1.0 ppm T_3	186.2 ± 6.2	(12)	84.8 ± 0.7	154.1 ± 9.8	(12)	73.2 ± 1.1	157.4 ± 8.7*	(17)	70.4 ± 1.1
Females									
Control	217.3 ± 11.6	(12)	82.1 ± 1.5	138.9 ± 9.3	(11)	67.9 ± 1.7	147.3 ± 8.3	(7)	64.3 ± 1.1
0.1 ppm T_3	200.1 ± 10.1	(12)	82.4 ± 1.4	199.6 ± 9.6***	(12)	73.9 ± 1.3**	155.4 ± 14.1	(5)	66.0 ± 1.8
0.3 ppm T_3	194.7 ± 12.0	(13)	81.2 ± 1.5	172.5 ± 7.4	(11)	72.8 ± 1.6*	138.8 ± 15.7	(8)	67.5 ± 1.7
1.0 ppm T_3	156.0 ± 6.22***	(13)	81.8 ± 0.8	166.0 ± 10.8	(11)	74.1 ± 1.2**	118.2 ± 14.4	(5)	64.6 ± 2.1

[a] Mean weight ± SEM with number of animals per group indicated in parentheses.
* Significantly different from the untreated control of that sex, $P < 0.05$.
** Significantly different from the untreated control of that sex, $P < 0.01$.
*** Significantly different from the untreated control of that sex, $P < 0.005$.

within treatment groups for unadjusted liver weights.

When examining the muscle data (Table II), essentially the reverse was observed. A significant positive treatment effect ($P < 0.05$) existed when the female unadjusted muscle weights were compared; the same trend (although not significant—$P > 0.05$) occurred within the males. When these weights were expressed as muscle weight per body weight, no significant differences ($P > 0.05$) were found except at the highest T_3 dosage where female SLD muscle weights were significantly ($P < 0.01$) depressed (Table II).

The second experiment assessed the effects of T_3 supplementation on humoral immune function and growth of the primary lymphoid organs as well as on general body growth. After examination of the body weight gain data this experiment again revealed a significant ($P < 0.01$) enhancement of growth only in the SLD strain and only at the intermediate T_3 dosage level (Table III). Examination of the T_3 treatments on primary lymphoid organ growth revealed several treatment effects. All growth data for the primary lymphoid organs are expressed on a per body weight basis to allow comparisons both within treatment groups and strains and to separate general growth effects from the specific effects of T_3 on the growth of these organs.

T_3 supplementation of the feed had a significant ($P < 0.01$) stimulatory effect on bursal growth in both K strain (0.01 ppm T_3) and ADW strain (0.1 ppm T_3) animals when compared to their respective untreated controls (Table III). Within the SLD strain, however, these treatments produced a definite dose-dependent depression of bursal growth with a maximal effect occurring at T_3 dosages of 0.10 ppm or greater. There were no strain differences ($P > 0.05$) in adjusted bursal weights between the untreated controls. Among the T_3-treated groups, bursal growth within the SLD was significantly decreased ($P < 0.01$) relative to the K control strain for a given dosage level.

A general trend of stimulated thymus growth due to T_3 supplementation was noted in all strains. Significant thymic growth enhancements, however, were observed only within the dwarf strains at either the 0.1-ppm (SLD) or 1.0-ppm (SLD and ADW) T_3

TABLE II. EFFECT OF T_3 TREATMENTS ON SLD LIVER AND MUSCLE GROWTH

Treatment	Liver wt (g/100 g body wt)		Pectoralis muscle wt (g/100 g body wt)	
	Male	Female	Male	Female
Control	3.69 ± 0.13[a] (7)	3.71 ± 0.15 (7)	8.76 ± 0.50 (7)	9.80 ± 0.54 (6)
0.1 ppm T_3	2.82 ± 0.17** (7)	3.16 ± 0.13** (8)	9.04 ± 0.42 (7)	9.83 ± 0.42 (8)
0.3 ppm T_3	2.58 ± 0.07** (8)	2.47 ± 0.08** (7)	7.92 ± 0.76 (8)	9.34 ± 0.96 (7)
1.0 ppm T_3	2.92 ± 0.14** (8)	2.85 ± 0.08** (8)	6.94 ± 0.36 (8)	6.40 ± 0.78* (8)

[a] Mean weight ± SEM with number of animals per group indicated in parentheses.
* Significantly different from the untreated control of that sex; $P < 0.01$.
** Significantly different from the untreated control of that sex; $P < 0.005$.

dosage levels (Table III). Thymic weights also differed between strains. The adjusted ADW thymic weights were significantly lower than the K control strain animals in all treatment groups. Within the SLD strain, only the adjusted thymic weights of the untreated control were significantly less ($P < 0.05$) than those of the K strain. At the highest T_3 dosage, the thymic growth of the SLD was stimulated to the extent that their mean adjusted thymic weight was significantly greater ($P < 0.05$) than that of K strain animals undergoing the same treatment.

With the addition of increasing amounts of T_3 to the diet, the antibody response of the SLD strain was increasingly depressed (see Fig. 1) resulting in a significant depression ($P < 0.05$) at the 1.0-ppm T_3 level. No effects on antibody production due to T_3 treatments were seen within either the K or ADW strains. When comparing the humoral immune responsiveness of the dwarf strains to the normal-growing K strain, the SLD produced significantly less ($P < 0.005$) antibody in all treatment groups. The anti-SRBC antibody production within the ADW strain, however, was not significantly different from the K strain within any of the treatment groups.

Finally, the effects of the T_3 treatments on serum levels of GH and the thyroid hormones (T_3 and T_4) were examined. The results are presented in Table IV. T_3 supplementation increased serum T_3 levels with significant increases occurring at the higher supplementation levels within all strains. Even following the exogenous addition of T_3, significant differences in serum T_3 levels still remained between the control K strain and the dwarf strains. For all treatment groups, the SLD strain was found to have significantly lower ($P < 0.001$) serum concentrations of T_3 than the K strain animals. The ADW strain also had generally lower T_3 levels when compared to the K strain, although to a lesser extent ($P < 0.05$) than was manifest within the SLD.

T_3 supplementation produced only minor effects on serum T_4 levels (Table IV). The only significant change ($P < 0.05$) in serum T_4 levels within any of the strains due to T_3 treatments was observed in the 0.01-ppm T_3 treatment group of the SLD strain. There were no significant differences ($P > 0.05$) in serum T_4 levels between strains for any of the treatment groups.

Supplementation with T_3 produced notable changes in serum GH levels within all strains (Table IV). The overall trend was toward depressed GH levels as dietary levels of T_3 increased, with maximal GH depression occurring in all strains at 1.0 ppm T_3. The major deviation from this trend was at the lower T_3 treatment dosages within the SLD strain. These treatments (0.01 and 0.1 ppm T_3) resulted in an elevation of serum GH levels with a significant increase ($P < 0.05$) in GH resulting from the lowest T_3 dosage level. This elevation is the only significant difference ($P < 0.05$) in GH serum levels between strains when the dwarf strains were compared to control K strain animals of a given treatment group.

Discussion. The effect of T_3 supplementation on SLD body growth seen in these studies is consistent with previous work. Decreased peripheral 5'-monodeiodinase activity found within this strain results in significantly depressed T_3/T_4 ratios (5) and T_4 supplemen-

TABLE III. EFFECT OF T_3 TREATMENTS ON BODY WEIGHT GAIN AND PRIMARY LYMPHOID ORGAN GROWTH

Treatment	Body wt gain			Bursal wt (mg/100 g body wt)			Thymic wt (mg/100 g body wt)		
	K	SLD	ADW	K	SLD	ADW	K	SLD	ADW
Control	370.5 ± 6.1[a]	241.2 ± 12.4	228.1 ± 14.0	121.44 ± 5.4	137.0 ± 9.6	104.1 ± 6.0	389.3 ± 26.3	309.1 ± 12.1	253.2 ± 19.0
0.01 ppm T_3	385.9 ± 14.2	273.2 ± 9.3	211.8 ± 11.2	155.6 ± 10.6**	109.1 ± 7.3*	122.2 ± 7.3	384.5 ± 29.1	302.8 ± 21.0	236.5 ± 15.9
0.1 ppm T_3	392.4 ± 9.4	316.0 ± 12.5**	260.2 ± 9.7	133.6 ± 5.3	85.9 ± 7.6***	146.4 ± 12.7**	447.5 ± 29.0	380.6 ± 28.3*	273.3 ± 18.5
1.0 ppm T_3	359.3 ± 13.9	246.4 ± 13.2	204.0 ± 18.9	122.9 ± 9.4	94.6 ± 4.6***	111.9 ± 6.6	390.5 ± 20.4	472.8 ± 25.0***	318.9 ± 11.1*

[a] Means of 15–18 animals per group ± SEM.
* Significantly different from the untreated control of that strain, $P < 0.05$.
** Significantly different from the untreated control of that strain, $P < 0.01$.
*** Significantly different from the untreated control of that strain, $P < 0.005$.

tation has only minor effects on growth and on serum T_3 levels (9). When this strain is supplemented with T_3, peripheral monodeiodination becomes unnecessary for the administered thyroid hormone to exert its full effect. As a result, at optimal levels, a highly significant stimulation of several growth parameters occurs within the SLD strain. Evidence supporting the conclusion that these effects are specific to the SLD strain (presumably as a result of increased sensitivity due to the initial deficiency condition) is supplied by the observation that T_3 supplementation had few effects on the same parameters in either the normal-growing K strain or in the autosomal dwarf strain. Similar investigations (15) using the same levels of T_3 supplementation for a fast-growing broiler strain also resulted in no enhancements of either growth or feed efficiency. This investigation (15) also reported the same negative effects on body weight gain at 1.0-ppm T_3 supplementation levels that were seen in both the K strain and ADW but not in the SLD strain.

The effects of T_3 supplementation on the growth of specific tissues as well as on general body growth were examined in these experiments. The same treatments that resulted in enhanced SLD body weight gain also produced significant increases in long bone growth (Table I). In mammals, one effect of T_3 stimulation is to increase somatomedin production by the liver (16). The SLD strain has been reported to have low somatomedin levels (7; L. Huybrechts, D. King, T. Lauterio, J. Marsh, and C. Scanes, unpublished data), and it is possible that the increased long bone growth which occurred in these experiments due to T_3 treatments was mediated by enhanced somatomedin activity. These same treatments were found to have no significant effect on long bone growth within either the K or ADW strains.

T_3 supplementation had no significant effect ($P < 0.05$) on unadjusted liver weights but when adjusted for body weights, the liver weights of all the T_3 treatment groups were depressed to a highly significant ($P < 0.005$) degree. SLD pectoralis muscle weights, however, when adjusted for body weight were significantly ($P < 0.01$) depressed only within the SLD female group receiving the highest T_3 dosage. This suggests that the general

TABLE IV. EFFECT OF T_3 TREATMENTS ON SERUM T_3, T_4, AND GH HORMONE LEVELS

Treatment	GH level (ng/ml)			T_4 Level (ng/ml)			T_3 level (ng/ml)		
	K	SLD	ADW	K	SLD	ADW	K	SLD	ADW
Control	381.0 ± 48.0[a]	274.0 ± 42.1	276.0 ± 55.3	38.4 ± 2.4	28.9 ± 3.4	31.5 ± 3.0	2.83 ± 0.20	1.56 ± 0.14	2.10 ± 0.14
0.01-ppm T_3	193.0 ± 33.9*	411.0 ± 51.2*	191.0 ± 32.0	34.7 ± 1.9	41.3 ± 3.8*	33.7 ± 3.9	3.03 ± 0.19	1.75 ± 0.09	2.75 ± 0.16*
0.10-ppm T_3	199.0 ± 50.4*	311.0 ± 53.7	151.0 ± 13.9*	30.2 ± 2.6	29.2 ± 2.8	25.5 ± 2.9	4.00 ± 0.27	2.41 ± 0.20	3.13 ± 0.18**
1.0-ppm T_3	82.0 ± 10.4**	51.8 ± 6.5**	64.9 ± 13.0**	31.7 ± 2.6	28.3 ± 3.3	24.2 ± 2.0	12.76 ± 1.12***	8.71 ± 0.83***	5.63 ± 0.35***

[a] Means of 10 animals/treatment per group ± SEM.
* Significantly different from the untreated control of that strain, $P < 0.05$.
** Significantly different from the untreated control of that strain, $P < 0.01$.
*** Significantly different from the untreated control of that strain, $P < 0.005$.

body growth stimulation observed within the SLD strain is largely due to increased muscle mass since the muscle weights appear to parallel body weights. Furthermore, it appears that the growth of all body organs is not being stimulated equally by these treatments (i.e., the liver weights remained essentially unchanged regardless of treatment).

In contrast, the T_3 treatments were found to have definite specific effects on the growth of both primary lymphoid organs within the SLD regardless of whether the organ weights were adjusted for body weight. Thymic growth was generally stimulated in all strains by the T_3 treatments with a significant dose-dependent stimulation occurring within both dwarf strains. This thymotropic effect of the thyroid hormones has been demonstrated previously (2, 17) and is consistent with the previously reported effect of T_4 treatments in these strains (9). The even greater thymic stimulation seen in these experiments due to T_3 treatments is readily accounted for by the increased biological potency of T_3 versus that of T_4 which was used previously (9).

Within the SLD strain, an inverse relationship appeared to exist between thymic and bursal growth, i.e., those treatments most stimulatory to thymic growth resulted in a dose-dependent depression of bursal growth. Whether this was a causal relationship remains to be determined. It is known, however, that both organs have a hormonal (18) as well as a lymphoid function, and it is possible that a form of feedback regulation exists (either directly or indirectly) between the primary lymphoid organs. A pertubation of such a balance by exogenous hormonal manipulation may then result in the stimulation of one at the expense of the other. Such a possibility is given further credence when the relationship between thymic and bursal growth in the K and ADW strains is examined. It should be noted that the lower T_3 dosages did produce significant enhancing effects on bursal growth in both the K and ADW strains. This observation suggests that T_3 alone does not necessarily exert adverse effects on bursal growth and may, at optimal concentrations, be stimulatory. These stimulatory effects of T_3 supplements on bursal growth occurred, however, at one T_3 dosage level of magnitude less than that which stim-

ulated thymic growth (Table III). Furthermore, when increasing T_3 dosages resulted in stimulated thymic growth, the stimulatory effects on bursal growth were lost. Finally, the differences observed between the SLD and the other two strains in the sensitivity of the bursa (thymus?) to exogenously administered T_3 may simply be due to a heightened overall sensitivity of the SLD strain to this hormone as a result of the T_3 deficiency which normally exists within this strain.

Evidence that treatments which affect the growth of a primary lymphoid organ may also affect the functioning of that organ is provided by an examination of the humoral immune responses of these animals. A direct relationship appears to exist between the ability of the various strains to produce anti-SRBC antibody and bursal growth. This is particularly apparent within the SLD strain. As bursal growth was depressed within the T_3-treated animals, the ability of this strain to mount an effective antibody response was also decreased. This dose-dependent depression in circulating anti-SRBC levels resulted in significantly less antibody production by those SLD chicks receiving the highest T_3 dosage when compared to untreated SLD controls. This may be contrasted to the results of previous experiments where growth hormone treatments enhanced both bursal growth and antibody production within this strain (9).

As discussed above, the depression of humoral immune responsiveness within the SLD strain by T_3 supplementation was an unexpected result. Given the functionally hypothyroid status (5) and the impaired antibody responsiveness (8) that has previously been reported for this strain, it was anticipated that perhaps the T_3 treatments not only would restore more normal growth characteristics but also would restore normal levels of humoral immune functioning to these animals. Other studies in both mammalian (17, 19) and avian (20, 21) species have demonstrated the importance of thyroid functioning for optimal immune development and function. Whether this depression in antibody production and bursal growth are due to a hormonal imbalance affecting the thymus and its immunoregulatory activities is a subject for further investigations.

The interactions and effects of T_3 treatments on serum T_3, T_4, and GH levels allow for several observations and conclusions. The relative insensitivity of the thyroid feedback control mechanisms to exogenously administered T_3 was an unanticipated observation. As T_3 serum levels increased, there was a definite tendency for T_4 levels to decrease which was apparent within both the K and ADW strains suggesting that some moderate negative feedback was occurring. However, even with four- to fivefold increases in serum T_3, T_4 levels were not significantly depressed in any of the three strains. This seeming insensitivity of the brain and pituitary to increasing serum T_3 levels is consistent, however, with current hypotheses on thyroid hormone feedback regulation (22) and with previous findings on the relative effectiveness of exogenous T_3 and T_4 in suppressing TSH release in the chicken (23) and in man (24). The brain and pituitary are sites of active intracellular 5′-monodeiodination of T_4 and thus are not dependent upon serum T_3 levels for feedback control. As a result, intracellular deiodination of serum T_4 provides at least half of the T_3 within the brain and pituitary that is involved in exerting feedback regulation. Thus, the serum T_3 becomes only a portion of the total T_3 available within these tissues. Other tissues, however, are primarily dependent upon the peripheral conversion of T_4 to T_3 and thus the serum levels directly reflect the relative amounts of the thyroid hormones available to them (22). Certainly the difference in responsiveness to serum T_3 levels between the hypothalamus/pituitary axis and the peripheral tissues seen in these experiments lends support to this theory.

These regulatory mechanisms, however, do not explain the increase in serum T_4 that was seen in the SLD strain after low-level T_3 supplementation. Following this treatment (Table IV), the observed increase in serum T_3 was modest and is occurring within a strain that is chronically T_3 deficient. A logical possible result of this low-level supplementation (which has already been shown to have several observable biologic effects at the tissue and organ level) is to enhance pituitary function. The supporting evidence for this interpretation is provided by the significant increases in both T_4 (presumably due to in-

creased TSH) and GH following low to intermediate T_3 treatments.

The second major effect of T_3 treatments on those serum hormones examined was the dose-dependent and highly significant depression of GH levels within all strains. The only exception to this observed trend was the initial rise in GH levels at the lower T_3 dosages described above for the SLD strain. At first inspection, this depression of GH would seem contrary to the reported stimulation of GH synthesis and release by the thyroid hormones (25, 26). In the chicken, however, recent findings have suggested an inverse relationship between thyroid functioning and growth hormone secretion (27). Furthermore, those studies that have shown increased GH release due to thyroid hormone treatments were generally those examining acute effects within hypothyroid animals (26, 28). The depression of serum GH in the studies reported here occurred only after serum T_3 levels were increased above those of the untreated controls. Indeed, in the functionally hypothyroid SLD strain, the lower T_3 treatments did increase GH levels in a manner consistent with similar observations in mammalian species (26, 28). Finally, the possible role of the somatomedins and/or somatostatin must be considered in interpreting the effects of T_3 treatments on GH serum levels. The increased T_3 serum levels may stimulate somatomedin release (16) and thereby depress GH directly or indirectly through stimulated somatostatin release. The fact that the depression of GH was delayed in the SLD strain is consistent with the observation of depressed somatomedins levels also in this strain (7).

A further observation may be made regarding the changes in hormone levels produced by T_3 treatments and the effect of these changes on growth and development parameters. Administration of the intermediate T_3 dosages produced a serum profile for the hormones examined within the SLD strain that was very similar to that seen in the untreated control K strain (Table IV), i.e., a normal endocrine environment. What makes this worthy of note is that the provision of this endocrine environment resulted in body weight gains and thymic weights for these SLD chicks which were not significantly different from the untreated K control (Tables I and III). It would thus appear that the lower T_3 dosages (0.01 and 0.1 ppm) are actually serving to establish a "normal" endocrine environment for the SLD strain and thereby allow the expression of full growth potential.

The major deviation from the SLD's return to normalcy due to T_3 supplementation is seen in the severely depressed bursal growth and presumably resultant depressed specific antibody levels. This is even more puzzling when one considers the elevated GH levels stimulated by the intermediate T_3 dosages. Previous studies have demonstrated that exogenous GH administration to this strain produces significant bursotropic effects (9). In those studies, however, there were also indications that T_4 treatments were somewhat antagonistic to the actions of GH and that GH may actually exert some control on the peripheral conversion of T_4 to T_3 (9). Similar observations were also made on the interrelationship between thymic and bursal growth, i.e., those treatments which stimulated thymus growth did not enhance bursal growth and vice versa (9). While a direct bursa-suppressing effect was not seen due to T_4 treatment, this is perhaps to be expected given the much greater thymic stimulation produced by T_3, the poor intrinsic ability of the SLD strain to convert T_4 to T_3, and the loss of any possible GH regulation of T_4 monodeiodination.

In summary, these experiments demonstrate the vital roles that the developmental hormones of the thyroid and pituitary may play not only in growth but also in humoral immune development and function. The importance of triiodothyronine versus thyroxine on a variety of parameters is strongly supported as is the interactive regulation of the thyroid hormones and growth hormone. Finally an interactive developmental and immunoregulatory relationship between the two primary lymphoid organs is suggested.

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