EFFECTS OF DIETARY IODINE ON SERUM THYROXINE AND THYROIDAL IODINE CONCENTRATIONS IN CHICKENS

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1968

Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1972

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ACKNOWLEDGMENTS

I would like to express my deepest graditude to Dr. W. S. Newcomer, my adviser, for his encouragement, enthusiasm and patience during the entire period of this work.

I would also like to take this opportunity to express my appreciation for guidance given to me by the other members of my committee: Dr. R. H. Thayer of Animal Sciences Department and Dr. J. G. Hurst of Physiological Science Department.

The technical assistance of Dr. D. Dunlay and Mr. L. A. Gale is gratefully acknowledged.

Thanks is given to the Animal Science Department for their contribution to this study.

The research herein reported was partially supported by funds from the National Institute of Health.

Finally, I wish to thank Mrs. W. S. Huang for her careful typing of the manuscript.

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CHAPTER I

INTRODUCTION

In recent years many investigations have been made in an attempt to elucidate the exact roles that iodine plays in the function of the thyroid gland. It has been reported earlier that the rate of thyroidal hormonogenesis *in vitro* and *in vivo* is influenced by iodine supply (18, 38, 39). Later, thyroid activity was found to vary inversely with the iodine content of both the diet (8, 11, 21, 23, 40) and the thyroid gland itself (3, 9, 10, 17, 31, 34). On the other hand, the versatility of the thyroid gland allows it to adjust its functional capacity to the changes in availability of iodine in diet or blood. Administration of iodine in excess or deficiency has been demonstrated to produce compensatory goiter in mammals (1, 14, 17, 31, 32, 37). However, it is generally accepted that normal thyroid function depends upon adequate intake of dietary iodine. Although the response of the thyroid gland to environmental iodine is remarkable, the mechanisms of the adaptation to dietary iodine levels are still unclear.

Some thyroid functions in birds have been shown to be quantitatively different from those in mammals. Goiter production is one of the paradoxical questions in that some investigators were able to produce goiter by feeding a diet deficient in iodine (24, 36) or one containing excess iodine (35), while others (15, 21, 22, 23) were not.

It was the purpose of this study to determine the length of time required for thyroid function to reach a state of equilibrium in birds fed various diets which differed only in their iodine content. Criteria of thyroid function studied in this experiment were thyroidal iodine concentration and serum thyroxine level. At the same time, it will be evidenced whether goiter production occurs in chickens under the conditions of dietary limitation or augmentation of available iodine.

CHAPTER II

LITERATURE REVIEW

Goiter and Thyroidal Iodine Content

Previous work has established that goiter production can be the result of either insufficient iodine supply or, less frequently, excessive iodine administration. In 1933, Levine, Remington and Kolnitz (14) developed the so-called Remington diet which produced goiter in the rat due to the fact that the diet was deficient in iodine content (1.5 μg of iodine per 100 grams of feed). On this diet goiters were produced in rats with a daily iodine intake of 0.14 microgram for 35 days of feeding. This finding has been confirmed in mammals by numerous investigators (1, 17, 31, 32). Astwood and Bissell (1) demonstrated that rapid thyroid growth, due to thiouracil treatment, resulted only when thyroidal iodine concentration had been depleted to low levels. Money, Rall and Rawson (17) examined the temporal sequence of thyroid function in rats fed a Remington diet (9 μg I/100 g of feed), and showed that thyroid enlargement did not become manifest until 3 months after beginning the feeding regimen. Furthermore, Studer and Greer (31) studied iodine-deficiency goiter in rats and found that a fall in thyroidal ^{127}I concentration was temporally related to the appearance of goiter. A later investigation by Studer and Greer (32) showed that goiters were produced within 7 days in rats fed a Remington diet (3 μg I/100 g of diet). They observed

a statistically significant increase in thyroid weight within one week after beginning the iodine-deficient regimen. However, Sinha, Anderson and Turner (30) recently failed to produce goiter in the rat by feeding a moderately low iodine diet (5 μg I/100 g of feed) for up to 3 months. From the data collected, they suggested that the rat thyroid had the capacity to increase its activity to compensate for any moderately low dietary level of iodine for at least 3 months by augmenting recycling mechanisms and thyroidal turnover of hormones. In addition, Halmi (11) and Van Middlesworth (33) were also unable to produce goiter in the rat by feeding the iodine deficient Remington diets.

Patton, Wilgus and Harshfield (24) were probably the first investigators to present evidence that goiter could be experimentally produced in chickens with a deficiency of iodine (14.5 μg/100 g of diet). Thyroids of chickens fed this diet were found to be 130% of the control thyroid weights at 6 weeks, 246% at 12 weeks and 294% at 18 weeks. Later, soybean meal has been found to be goitrogenic in chickens (36). However, Newcomer (21, 22, 23) was unable to confirm the findings of these investigators. When chickens were fed diets which contained 1, 8, or 10 μg of iodine/100 g of feed for 6-10 weeks, the thyroid weights remained normal.

Observation on total thyroid iodide content in intact and hypophysectomized rats led Vanderlaan and Caplan (34) to suggest that the relationship between iodide-concentrating capacity and total thyroidal iodine concentration was reciprocal. Greer, Grimm and Studer (9) concluded that a preferential synthesis and secretion of T_3 over T_4 in severe iodine deficiency occurs in rats. This is

an important adaptive mechanism for maintaining a euthyroid state in the face of inadequate iodine supply. Data obtained by Bray (3) and Greer and Rockie (10) showed that iodine depletion of rat thyroid increased the sensitivity of the thyroid to the goitrogenic effect of thyrotropin and produced a high T_3/T_4 ratio. However, Rosenberg, LaRoche and Dimick (27) proposed that chickens are able to adapt to the low availability of iodine (3 μg I/100 g of feed) by adjusting their thyroxine secretion rate to a minimal level.

Experimental evidence in support of the iodine excess hypothesis of goiter has been exhaustively reviewed by Wolff (37). Excessive exogenous iodide has been thought to affect thyroid function by blocking hormonal synthesis temporarily (18, 38, 39), the so-called Wolff-Chaikoff effect, and by reducing hormonal secretion from the gland (8). Wolff (37) concluded that chronic ingestion of iodide or iodide-generating organic compounds in amounts of ten or more times the daily requirements for hormone biosynthesis leads to iodide goiter in certain individuals.

Wheeler and Hoffmann (35) reported that goiters were observed in the chick fed a ration with high dietary iodine content (64.5 mg I/lb of diet). However, Newcomer (23) and Mayberry and Hockert (15) were unable to confirm this observation of Wheeler and Hoffmann. Newcomer (23) fed a iodine-enriched diet containing 50 mg I/lb of feed to chicks for a period of 4-6 weeks and found that their thyroids were similar to those of the control birds in size but not in the shape of follicle cells. The effect of excess iodide in the drinking water on thyroid function of chicks was studied by Mayberry and Hockert (15) and they

reported that no goiter production was found in four breeds of chicks fed up to 0.5% (w/v) KI in water for periods of up to six months.

Serum Thyroxine Concentration

Bumgardner and Shaffner (4) found that the plasma protein-bound iodine (PBI) value in normal chicks was 1.06 microgram percent. Mellen and Hardy (16) reported a similar value (1.33 μg%) in chickens and noted that the PBI level in fowls was much lower than that in mammals. They also stated that PBI is not a good criterion of thyroid activity in the fowl, since experimental alteration of thyroid activity did not produce changes in blood PBI level in chickens. The low levels of avian PBI was further confirmed by Kelsey, Gullock and Clausen (13), who found that PBI in a hawk was 1.6 μg%.

Rosenberg, Goldman, LeRoche and Dimick (26) demonstrated that iodine supplementation of a low-iodine diet (14 μg I/100 g of diet) caused a large increase in circulating ^{127}I in both rats and chickens. They observed a significant increase in circulating $PB^{127}I$ of rats but only an insignificant increase in circulating $PB^{127}I$ of chickens. Thyroid content of ^{127}I in both rats and chickens was increased 3- to 5-fold by the iodide supplement (2100 μg/100 g of food). High iodide supplementation caused only a minor increase in serum $PB^{127}I$ of the chicken, but a large increase in circulating total ^{127}I. Studer and Greer (31) discovered an interplay between autonomous and thyrotropin-dependent intrathyroidal mechanisms that allows maintenance of a normal level of circulating thyroid hormone in an iodine-deficient state until the body iodine pool becomes too severely depleted.

Serum T_4 concentration, using the competitive protein binding

technique of Murphy (19), was measured in an attempt to replace PBI as an index of thyroid function. Murphy (19) reported that euthyroid human beings had a T_4 level of 6.2-7.8 μg%, while hypothyroid subjects had 2.9 μg% and in hyperthyroid subjects the level was 14.8 μg%. Later, Nathanielsz (20) made a sequential study of plasma thyroxine in the lamb, which had a T_4 level of 5.7-7.7 μg% during the period from 28 to 61 days of age. Dalrymple and Utiger (6) modified the T_4 competitive binding assay which allowed the use of serum samples as small as 50 microliters and found that serum T_4 concentration in normal rats averaged 3.8 μg%. The PBI and serum T_4 of 16 selected vertebrated species were compared by Refetoff, Robin and Fang (25), who reported the total T_4 in Leghorn chickens was 1.4 μg%. They noted that adult birds had moderately raised free T_4 levels when compared to humans and most warn blooded vertebrates; a fact which might be responsible for higher heart rates, oxygen consumption and elevated body temperature in birds as compared to most mammals. In contrast to these investigators, Sadovsky and Bensadoun (28) reported that serum thyroxine values ranged from 3.6 to 5.6 μg% and total plasma hormonal iodine values ranged from 4.8 to 11.9 μg% by using chromatography techniques. The total hormonal iodine values are several-fold higher than those reported using a PBI technique. A diurnal rhythm of plasma iodohormones was also observed.

CHAPTER III

MATERIALS AND METHODS

Management Procedure

A basic, low-iodine diet was prepared, the composition of which is shown in Table I. Different amounts of NaI were added to the basal ration to produce a range of diets which differed only in iodine content. The calculated iodine contents of these diets are shown in Table II. Five groups of White Leghorn cockerels were fed from day-old with these five different diets. The group number corresponds to the number of the diet fed. Distilled water was given throughout the experiment. Birds were housed in floor pens at the Poultry Farm of the Animal Sciences Department, Oklahoma State University.

Tissue Collection

From day-old up to day 56 in some groups, birds were withdrawn serially from each of the five groups and killed by decapitation. Ten birds in each groups were killed at one time. Blood was collected and serum was obtained for determination of thyroxine (T_4). Thyroid glands were dissected out, trimmed clean and weighted immediately. Thyroids were frozen until time of assay of iodine content. A rapid method of obtaining a good yield of serum without centrifugation was found incidentally during this study; the collected blood in the test

TABLE I

BASAL RATION

Ingredients	Percentage
Corn starch	38.72
Polyethylene (ground)	4.00
Soybean meal (50% protein)	50.00
Tallow (feed grade)	2.00
Dicalcium phosphate	4.00
NaCl[1]	0.50
Manganese sulfate	0.013
Vitamin mixes	
VC-60 (no trace minerals)[2]	0.50
VC-60A[3]	0.25
DL methionine	0.03
TOTAL	100.013

1. Baker, reagent grade, Iodine less than 0.002%.

2. VC-60—adds per pound of finished ration: vitamin A, 8,000 U.S.P.; vitamin D_3, 1,200 I.C.U.; vitamin E, G.I.U.; vitamin B_{12}, 0.008 mg; riboflavin, 4 mg; niacin, 32 mg; pantothenic acid, 8 mg; choline chloride, 500 mg.

3. VC-60A—adds per pound of finished ration: pyridoxine, 8 mg; biotin, 0.3 mg; thiamin, 12 mg; folic acid, 2 mg; inositol, 50 mg; para amino benzoic acid, 4 mg; ascorbic acid, 10 mg.

TABLE II

CALCULATED IODINE CONTENT OF DIETS

Diet	Iodine Content μg/100 g Diet
I	1
II	5
III	10
IV	100
V	1000

tube was ringed around the inner wall of the test tube with a clean applicator stick (Puritan).

Determination of Thyroidal Iodine Concentration

The stable iodine concentration in thyroid was determined colorimetrically by iodine catalysis of the oxidation reduction reaction between ceric sulfate and arsenious acid in a method devised by Bates (2).

Preparation of Reagents

1. Standard Solutions

   A. Stock solution I (100 μg I^-/ml)

   Dissolved 16.85 mg dried KIO_3 (Mallinckrodt Anal. Reag. Grade) in glass-distilled water to a total of 100 ml. This solution

can be stored at 4°C for a period of at least 4 months.

B. Stock solution II (1 μg I^-/ml)

One ml of stock solution I was diluted to a total of 100 ml with glass-distilled water.

C. Working solution (0.1 μg I^-/ml)

Ten ml of stock solution II were diluted to 100 ml with glass-distilled water.

2. 0.1 N Ceric Ammonium Sulfate

Two gm $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (G. F. Smith Chem. Co.) and 9.0 ml of conc. H_2SO_4 (Baker Anal. Reag. Grade) were added to glass-distilled water to make 100 ml. In order to produce a similar standard curve, this color reagent was used within 2 days after preparation.

3. 0.5% Sodium Chromate

Five hundred mg of $Na_2CrO_4 \cdot 4H_2O$ (Mallinckrodt Anal. Reag. Grade) were dissolved with glass-distilled H_2O to a total volume of 100 ml.

4. 0.2 N Arsenious Acid with Sodium Chloride

Into a 1000 ml volumetric flask, 9.891 gm AS_2O_3 (Mallinckrodt Anal. Reag. Grade), 7.0 gm NaOH (Mallinckrodt Anal. Reag. Grade), and glass-distilled H_2O were added to a volume of about 100 ml, and allowed to dissolve. Then 200-250 ml glass-distilled H_2O and 46.5 ml conc. H_2SO_4 were added and the flask was cooled to room temperature.

Five gm NaCl were added and made to 1000 ml with glass-distilled H_2O.

Complete reduction will occur in 5-10 minutes with this reagent.

5. Chloric Acid Reagent

Into a 3000 ml round-bottom flask, were added 500 gm $KClO_3$ (Mallinckrodt Anal. Reag. Grade) and 900 ml glass-distilled water. The mixture was subjected to reflux until the $KClO_3$ was dissolved, or nearly so. Then 375 ml of 70% $HClO_4$ (G. F. Smith Chem. Co.) were added cautiously with continual stirring to help disperse the heat of reaction. After incubation in the refrigerator for 24 hrs., the mixture was filtered and 1000 ml of yellow liquid which becomes colorless in 4-6 days were obtained.

Digestion of Thyroids

The thyroids were kept frozen until they were subjected to digestion. The gland or glands were placed into 180 ml beakers which contained 2.0 ml chloric acid reagent per 10 mg thyroid tissue. Two drops of 0.5% sodium chromate per 10 mg tissue were added. The beakers were covered with watch glasses and placed on a hot plate which was pre-set at 139°C for slow, steady boiling. When most of the water had boiled off and white fumes appeared, the watch glasses were removed and the evaporation was continued until 0.1-0.2 ml of the digest remained. At this point, 0.5 ml of chloric acid was added to prevent loss of iodine and to complete digestion. The mixture was evaporated to 0.1-0.25 ml. The beakers were removed from the hot plate and allowed to cool to room temperature. Twenty ml of glass-distilled water were added to make the digested thyroid solution.

Standards were digested and treated in the same manner as the samples. The digestions were carried out under an exhaust hood, since the white fumes of chloric acid are noxious.

Colorimetric Quantitation of Iodine

1. Four ml of standards or certain amounts of unknown samples were pipetted into 18 x 150 mm test tubes (Pyrex). The amount of unknown or digested thyroid solution pipetted depends on the iodine content of the sample which can be roughly tested prior to the assay. Four ml of glass-distilled water were used as a blank.
2. Total volume in each tube was made to 4 ml by adding glass-distilled water if necessary.
3. Two ml 0.2 N arsenious acid with NaCl were added and mixed well with a Super-Mixer (Matheson Scientific Co.). The mixture then was allowed to set 10 minutes for complete reduction of the iodate.
4. The spectrophotometer (Coleman Instruments, Inc.) was allowed to warm up at least 10 minutes prior to its use.
5. One ml of 0.1 N ceric ammonium sulfate was added to each tube of the series of tubes at 15-second intervals with one hand. The tubes were shaken immediately after adding this reagent with the other hand.
6. Spectrophotometer readings (at 425 microns) were made precisely 10 minutes after adding the color reagent. The readings were made at a rate of one tube per 15 seconds. It is important to have a constant temperature throughout the readings.

Calculation of Thyroidal Iodine Concentration

A proper dilution factor was used for each sample. The amount of iodine in the tissue sample was determined from the standard curve and the iodine concentration in thyroid (μg/mg) was calculated by the following formula:

$$\frac{\text{Reading from standard curve } (\mu g) \times \text{dilution factor}}{\text{Thyroid weight (mg)}} = \frac{\mu gI}{mg}$$

Determination of Serum Thyroxine Level

Concentration of thyroxine in serum was estimated in duplicate by competitive displacement analysis which was described by Murphy (19) in 1965 and modified by Dalrymple and Utiger (6). The present analysis of T_4 employed (with slightly modifications) the combined techniques of the two former mentioned laboratories.

Preparation of Materials or Solutions

1. Stable Thyroxine Solutions

   Stock I (50 μg/ml)—Five mg of L-T_4 (free acid, Sigma Chem. Co.) were dissolved in 0.6 ml propylene glycol plus a few drops of 1 N NaOH and diluted to 100 ml with 95% ethanol.

   Stock II (5 μg/ml)—Ten ml of Stock I were diluted to 100 ml with 95% ethanol.

   Working Standard Solution (50 mμg/ml)—One ml of Stock II was diluted to 100 ml with 95% ethanol.

   Standard T_4 solutions were stored at $4^{\circ}C$ for the period of 2 months.

2. ^{125}I—Thyroxine Solution

   ^{125}I—Thyroxine (activity 328 microcuries/ml) in 50% propylene glycol was obtained from Abbott Laboratories, North Chicago, Illinois.

3. Resin

   Rexyn 201 ($Cl\text{-}SO_4$) (Fisher Scientific Co.), medium porosity, was used. After soaking overnight in barbital buffer (pH 8.6), the

resin was rinsed in buffer several times until the pH was approximately 8.6. The supernatant was discarded and the resin was dried at 90°C and sieved; the particles larger than No. 20 mesh and smaller than No. 40 were discarded.

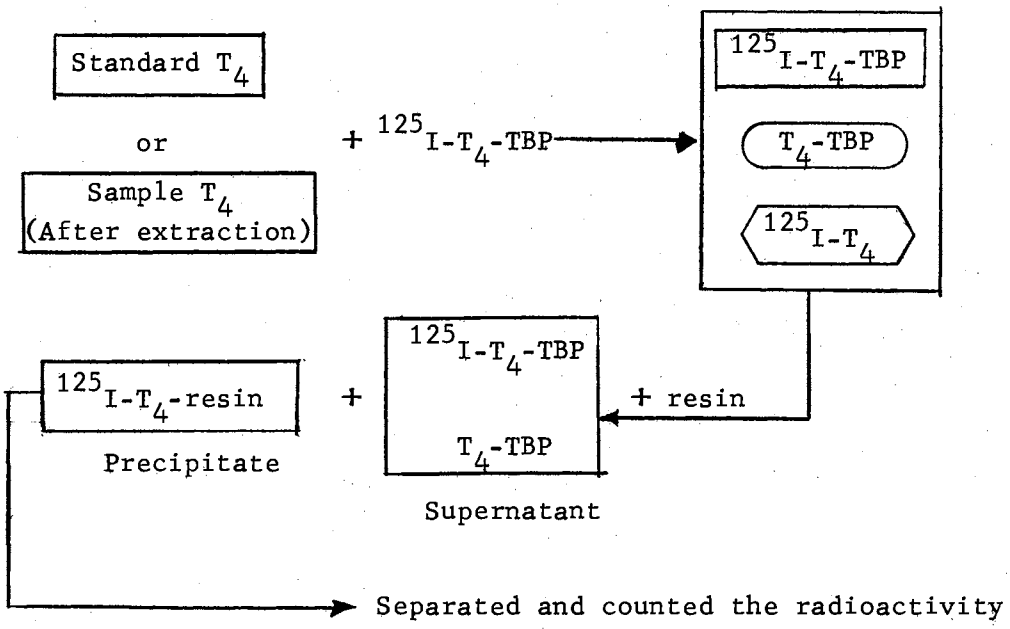
The weight of 20 separate spoonsful of resin gave a mean of 238.46 ± 2.85 mg (S.D.) indicating that the amount of resin could be accurately controlled by use of the spoon.

4. ^{125}I-thyroxine binding protein (^{125}I-T_4-TBP) solution

This solution was prepared by combining in a volumetric flask (1) normal human serum, 3.5 ml; (2) propylene glycol, 2.5 ml; (3) 1% phenol, 2.5 ml; (4) ^{125}I-T_4, approximately 10,000 cpm and 0.2 to 0.4 mμg T_4 per milliliter of ^{125}I-T_4-TBP solution; and (5) 0.5 M barbital buffer, pH 8.6, to a total of 500 ml.

Assay Methodology

Competitive displacement analysis was used as shown in the following diagram:



Assay Procedure

Fifty μl of serum were added in duplicate to 800 μl of 95% ethanol in 3-ml disposable tubes. After complete covering with parafilm paper, the suspension was mixed well on a Super Mixer and centrifuged at 3,000 rpm for 15 minutes. Three hundred μl of the supernatant were transferred to counting tubes. Standards (0, 0.5, 1, 2, 3, mμg of stable T_4) were prepared and added to similar tubes, in duplicate. All tubes were evaporated to dryness in a 45°C water bath with a gentle air stream.

For accuracy, tubes were run in groups of 10 at one time in the next essential steps. To each of 10 tubes was added 1 ml of ^{125}I-T_4-TBP solution. The tubes were shaken gently at 45°C for 8 minutes and then transferred to an ice bath where they were cooled for 10 minutes. After the tubes (in a rack) were removed from the ice bath, one spoonful of resin was immediately added to each tube. The rack was shaken vigorously with a horizontal motion for exactly one minute and 3 ml of 4°C barbital buffer were rapidly added with an automatic syringe, then mixed by shaking for 15 seconds. The resin settled quickly and the supernatant was aspirated.

Following 2 additional cooled buffer washes, the tubes containing resin were counted in a well-type, crystal scintillation detector (Baird-Atomic, Inc.). Tubes containing 1 ml of ^{125}I-T_4-TBP solution were counted as total radioactivity. The radioactivity of the resin was expressed as a percentage of the total.

Calculation of Serum Thyroxine Concentration

The quantity of T_4(mμg) in the dried ethanol extract was determined from the standard curve and converted to micrograms per 100 ml serum (μg%) by a factor of 17/3 (Dalrymple and Utiger, 1970) as shown in the following formula:

$$m\mu g \text{ (in 300 } \mu l \text{ of ethanol extract)} \times \frac{850}{300} \text{ (changes fraction of sample analyzed to the original 50 } \mu l \text{ of serum)} \times \frac{20 \times 100}{10^3}$$

(changes mμg/50 ml to μg/100 ml serum)

$= 17/3$

Determination of Recovery

1. Recovery of $^{125}I\text{-}T_4$

   In order to test the percentage of extraction of thyroxine into alcohol, 300 μl of $^{125}I\text{-}T_4$ in 95% ethanol were added to each of 12 tubes, and evaporated to dryness. Fifty μl of chicken serum were added to each tube, incubated and shaken gently at 45°C for 8 minutes. The radioactivity was counted. After adding 800 μl of ethanol, each tube was mixed and centrifuged at 2,000 rpm for 5 minutes. Three hundred μl of supernatant was transferred to another counting tube and counted.

$$\% \text{ recovery} = \frac{\text{cpm supernatant} \times \frac{85}{30}}{\text{cpm added}} \times 100$$

2. Recovery of nonradioactive thyroxine

   This procedure was used to test if there was any loss of T_4 other than that associated with the extraction step. 1 ml of stable T_4

was added directly to ethanol extracts of serum which were then dried and assayed.

$$\% \text{ recovery} = \frac{\text{Total } T_4 \text{ in m}\mu\text{g} - T_4 \text{ of ethanol extract in m}\mu\text{g}}{T_4 \text{ added in m}\mu\text{g}}$$

The test for percentage recovery of T_4 showed that for radioactive T_4 was 101.65 $\pm$ 0.98 (mean $\pm$ S.E.) percent and for stable T_4 was 99.89 $\pm$ 1.85 percent (Table IX in the appendix). There was no correction necessary for T_4 values obtained experimentally in this study, since the recoveries were almost 100 percent.

CHAPTER IV

RESULTS

The results obtained from the five treatments where the effects on iodine metabolism of five calculated levels of dietary iodine (namely: 1, 5, 10, 100 and 1000 μg I/100 g of diet) were studied are summarized in Figures 1, 2, 3 and 4. Each point is the mean for ten chickens. The raw data and means ± S.E. (standard error) will be found in the appendix; S. E. are not shown on the graphs for the sake of clarity.

Body Weight and Growth Rate

The mean body weights of birds in group I and group II were similar and the apparent growth rates appear to be normal (Fig. 1). Growth rates of birds in group III were less than those of birds in I and II up to 23 days at which time mean body weight of birds in III was 65% of that of birds in I and 76% of that of birds in II. Body growth rates of birds in group IV and group V were much slower than those of birds in I and II. At day 31, the mean body weights of birds in IV and V were smaller than those of birds in I and II; this difference was statistically significant ($p < 0.001$ by "Student's" t-test).

These data indicate that normal growth rates occurred at the dietary iodine level between 1 and 5 μg/100 g of diet. It is apparent

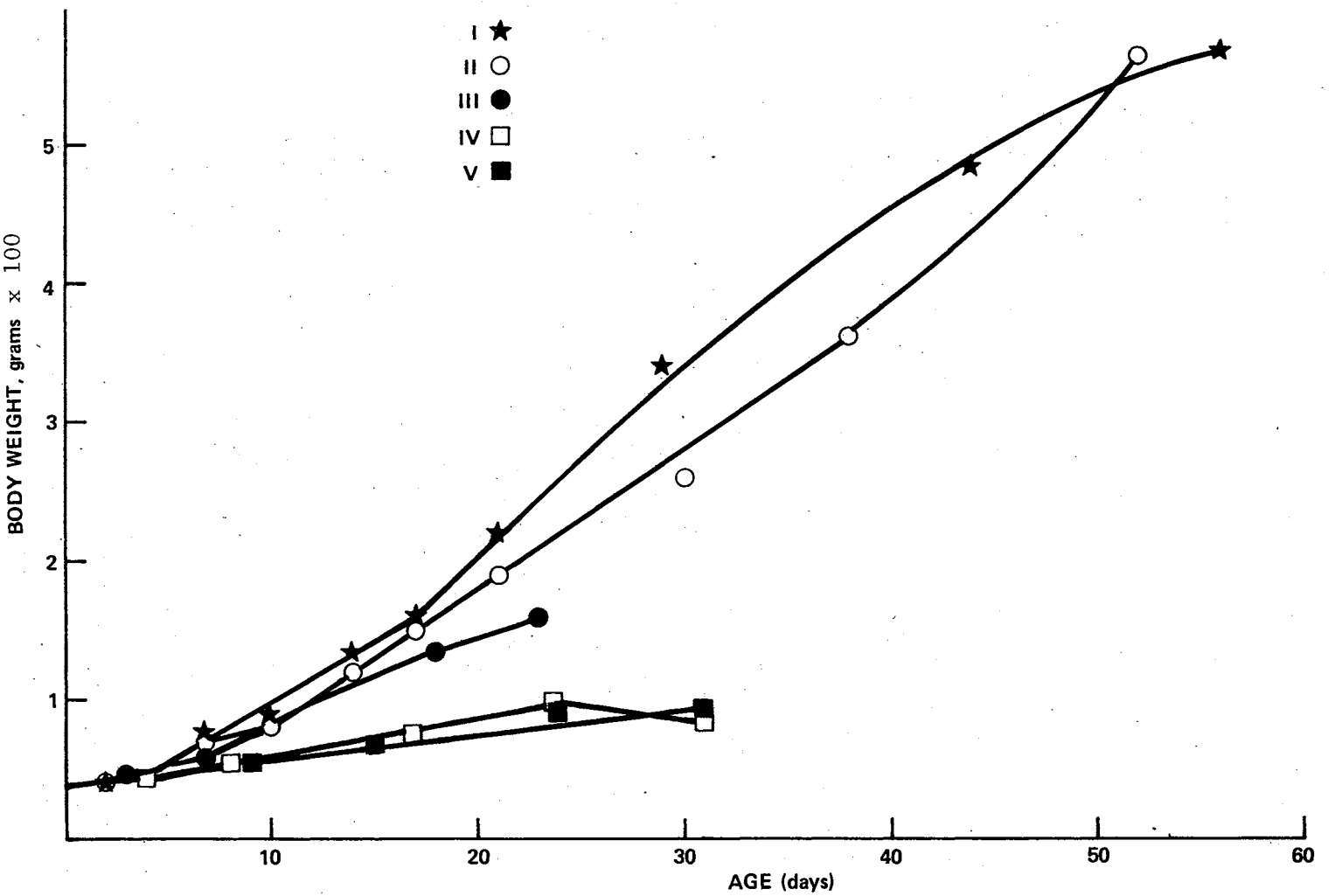


Figure 1. Body Weight at Different Ages of Five Groups of Chicks

that body growth was markedly depressed by dietary iodine higher than 100 μg/100 g of diet during the first month after hatching.

Thyroid Weight

In Fig 2 the mean thyroid weights (one gland per bird) are expressed in mg per 100 g of body weight. It appears that in all five groups, thyroid weight decreased gradually from hatching until two weeks of age, then separated into a pattern of three groups which differed significantly one from another. The thyroid weights in III, IV and V fell continuously and maintained a level around 2 mg/100 g of body weight at the end of the first month. After 14 days, the mean thyroid weights of birds from II did not change beyond the range of 2.5-3.5 mg/100 g of body weight and occupied the middle position of the 3-group pattern.

A conspicuous thyroid enlargement or goiter was not observed in the present study. Although the mean thyroid weight in I did rise sharply and reach 4.70 mg/100 g body weight after the age of 2 weeks, it failed to increase or maintain this high level continuously. It fell to about 3.0 mg/100 g of body weight at the end of this study (56 days).

Stable Iodine in the Thyroid

The mean stable iodine concentrations in the thyroids of the five groups of chickens in relation to the age of the chickens are shown in Fig. 3. The mean iodine level at hatching was 1.03 ± 0.1 (S.E.) μg/mg of thyroid. During the first week there was no regular pattern of response of thyroidal iodine to dietary iodine. However,

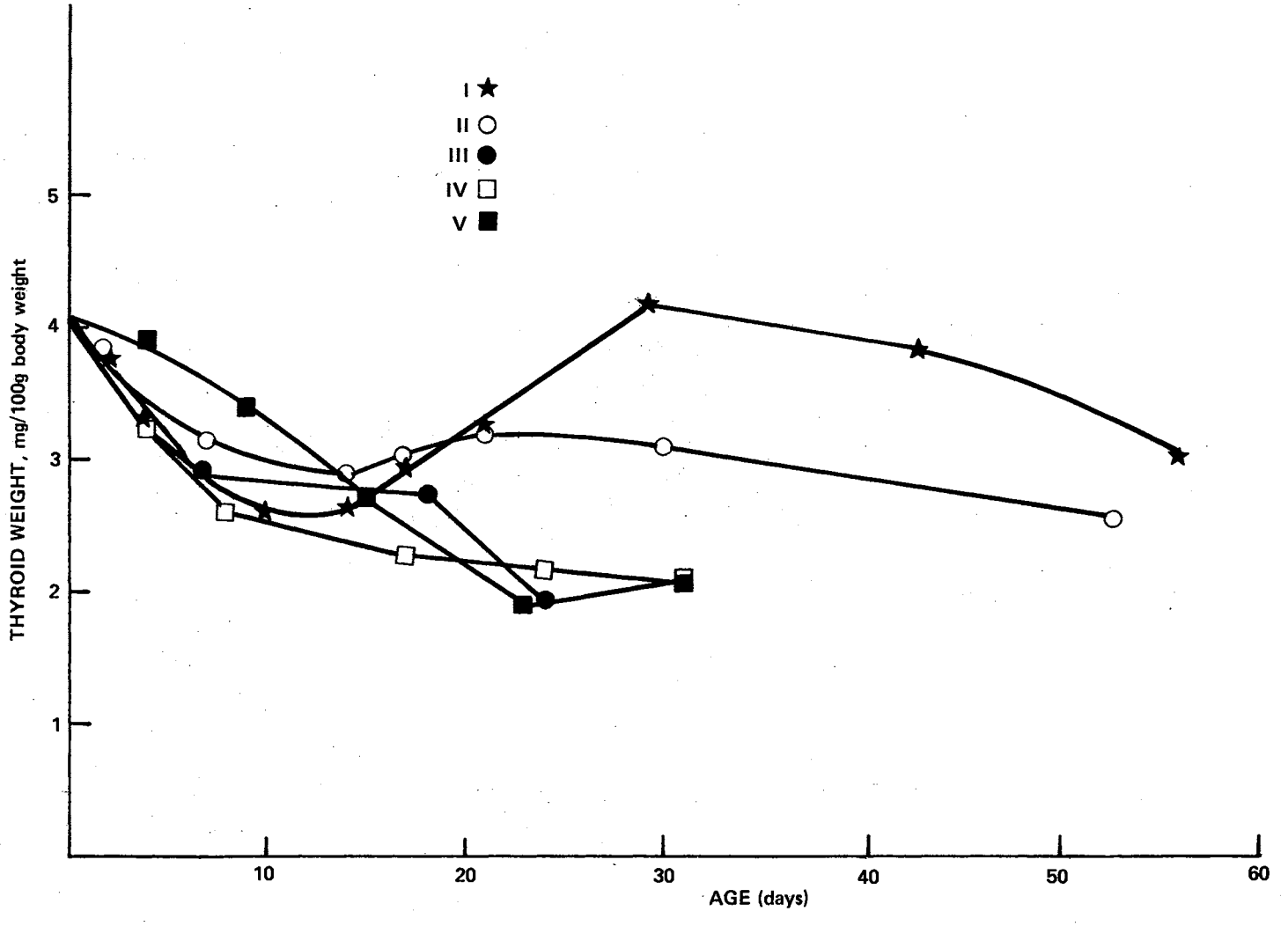


Figure 2. Thyroid Weights (mg%) of the Five Group Chicks

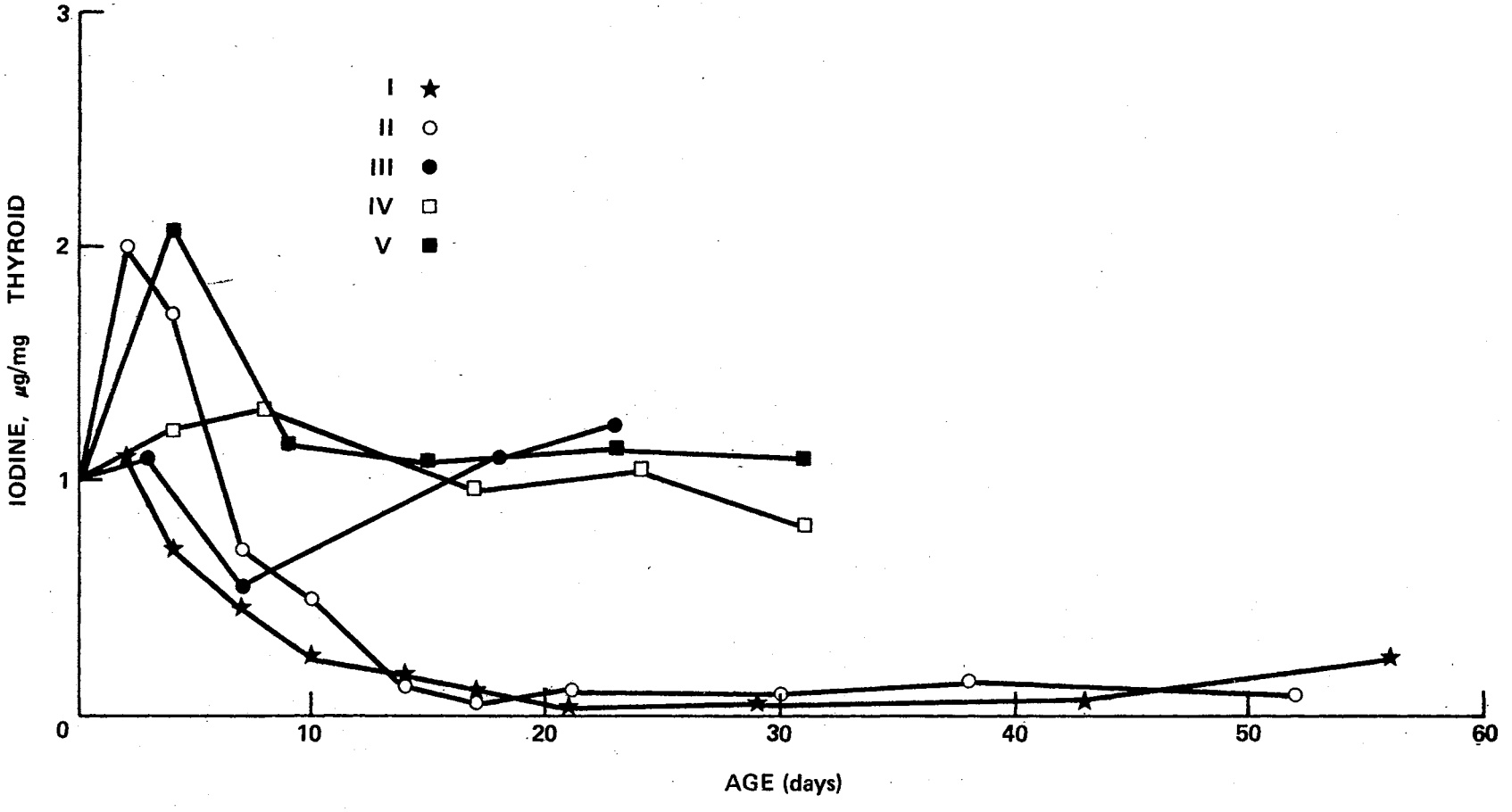


Figure 3. Thyroidal Iodine Content at Various Stage of Age

after 14 days the thyroidal iodine concentrations did exhibit a pattern which was associated with dietary iodine in that two groups separated significantly from the others by a wide gap: groups I and II maintained an extremely low level (less than 0.3 μg/mg of thyroid) while groups III, IV and V remained at a relatively high level which is close to the level at hatching (about 1.0 μg/mg of thyroid).

The data also indicate the important fact that the equilibrium of thyroidal iodine concentrations with dietary iodine contents had been reached two weeks after hatching in each group of birds studied.

Serum Thyroxine

The mean concentration of serum thyroxine (T_4) at hatching was 8.21 $\pm$ 0.91 μg/100 ml serum (μg%) as shown in Fig. 4. Drastic fluctuations of mean serum T_4 levels can be seen during the first week of age. However, from day 10 onward, serum T_4 of birds in I and II decreased and finally reached an equilibrium condition at 2-3 μg% between 40 and 56 days. Thyroxine level of birds in III and V was maintained in the range of 5-7 μg% from day 3 to 31. They attained equilibrium earlier than birds in I and II. The birds in IV had relatively high thyroxine concentrations in the first two weeks. Equilibrium was attained in this group at 4-6 μg% during the period from day 20 to 31.

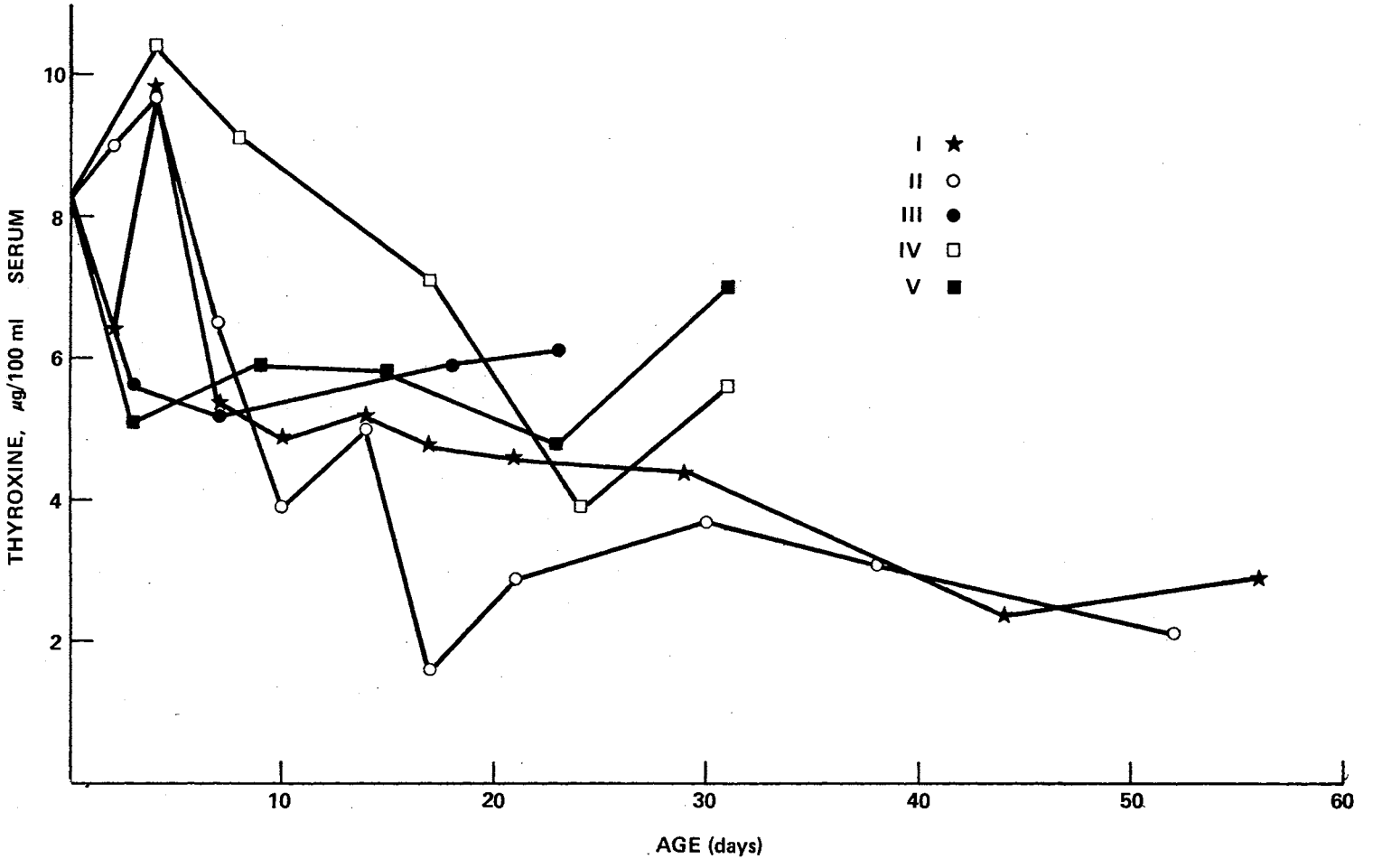


Figure 4. Serum Thyroidal Level Vs Age of Five Groups of Chickens

CHAPTER V

DISCUSSION

Body Weight and Growth Rate

As revealed in Figure 1 wherein mean body weights of birds in the various groups (I-V) were plotted as a function of time, the apparent growth rates of birds in I and II appear normal. The growth rates of birds in IV and V are comparatively slow. The mean body weight of birds in III at the age of 23 days was 65% of that in I and 76% of that in II.

It has now been established beyond doubt that the thyroid hormone is essential for growth during early life in mammals and birds (29). Further, differences in growth rates of different strains of chickens have been correlated with thyroid secretion rates; the faster growing strains have a higher thyroxine secretion rate than the slower growing strains (7). In this study, the normal growth rates of birds in I and II might be due to enhanced thyroxine secretion rates under the condition of relatively low availability of dietary iodine. Thus one would suspect that thyroxine turnover rates are also increased above normal to maintain the normal growth rates, since the circulating thyroxine was equilibrated at low levels in the birds of I and II.

Cameron and Carmichael (5) administered large doses of thyroxine (100 μg/100 g of body weight) to rats over 2 months and found that growth as a whole was retarded presumably because of the stimulation of

catabolic processes. The low growth rates in chickens of IV and V in this study might be ascribed to the high serum thyroxine levels as in the rat. However, the birds in III had similar amount of serum thyroxine as the birds in IV and V, but their growth rates were not as slow as that of the birds in IV and V. Therefore, it is likely that a certain high level of inorganic iodide in the blood is also involved in retarding the body growth of birds in IV and V, since the inorganic iodide concentrations in the blood of birds in IV and V are assumed to be higher than that of birds in III.

Thyroid Weight

The data (Figure 2) clearly indicate that thyroid weight expressed as mg per 100 g of body weight (mg%) in all five groups decreased gradually during two weeks of age from an initial value of 3.16 mg%. This decrease can be ascribed to the disproportional growth of thyroid and body, and not due to dietary iodine. As the data show (see appendix), the thyroid weights remained fairly constant but the body weights increased gradually during the first two weeks after hatching; consequently, the ratio of thyroid weight to body weight decreased naturally.

After the age of two weeks, the effect of administering different concentrations of dietary iodine on thyroid weight resulted in a pattern of three groups: thyroid weights in II remained relatively constant, while those in I were higher and those in III, IV and V were lower. The lower thyroid weights of birds in III, IV and V (as compared to those of I and II) might be due to less stimulation of the thyroid gland by thyrotropin (TSH); the TSH being depressed

by the high level of circulating thyroxine. The rapid increase in thyroid weights in birds of I immediately after 14 days is due presumably to the augmented TSH stimulation caused by the low serum thyroxine level. But the fact that thyroid weights remained fairly constant in birds of II, which had a similar amount of thyroxine in the blood as that in birds of I is not understood.

Goiter production has been demonstrated in mammals by numerous investigators (1, 14, 17, 31, 32) and in birds (24, 36) in compensation for limited availability of iodine. In the present study, goiter production did not occur, although the lowest dietary iodine content (1 μg iodine per 100 g of diet) used for I in this study is as low as that of the Remington diet which has been shown to produce goiter within only 7 days in rats (32). The present results are in agreement with those investigators (21, 22, 23), who found no goiter with similar amount of iodine-limited diets in chickens.

The fact that goiter was not produced in the chickens of groups I and II on a dietary level of iodine which does produce goiter in rats (32) can be associated with the observation that the T/S ratio of birds on a low-iodine diet can reach 800 or above (23) in contrast to the value of only 250 in rats (32). Thus it can be concluded that chickens adapt to low dietary iodine by a tremendous elevation in T/S ratio due to increased iodide transport and less by an enlargement in gland size in contrast to the reverse in rats. The ability of the thyroid glands to increase their concentrating mechanism of iodine is due presumably to the increased sensitivity of the gland to the effect of TSH as previously stated (10). Another factor which enables the thyroids to increase its trapping mechanism is the augmented circulating TSH

via the negative feedback mechanism. Since circulating thyroxine levels were low in birds of I and II (see Figure 4), presumably the TSH level in blood of these groups was high.

Although ingestion of massive amounts of iodine was reported to produce goiter in mammals (37) and in birds (14), it is evident from this experiment that the augmented amounts of iodine in the diet up to 1000 μg/100 g of diet did not produce any tendency toward enlargement of the chick thyroid (Figure 2). This lack of effect of high iodine on the thyroid is likely not due to an absence of elevated blood inorganic iodine in the groups which were fed high-iodine diets, because iodide is very water soluble and rapidly absorbed from the intestine (12). However, this study confirms other observations (15, 23) which showed no goiter production by feeding excess iodine in chickens.

It is possible that no goiter in birds with high iodine supply is due partially to high circulating thyroxine levels which suppress TSH secretion from the pituitary gland. If high iodine diets do indeed produce goiter, then the highest dietary iodine content (1000 μg/100 g of diet) used in this experiment was not high enough to cause goiter production.

Stable Iodine in Thyroid

The most striking effect of dietary iodine on thyroid activity can be seen in Figure 3. The stable iodine concentrations in the thyroid obviously became stablized and exhibited a pattern consisting of two constant levels or "steady states," which were separated by a wide gap after 10 days of age in the chick.

Iodine concentrations in thyroids of birds in III, IV and V equilibrated at approximately 1 μg I/mg of thyroid, which is the level in thyroids at hatching; while those of birds in I and II reached a "steady state" at about 0.15 μg I/mg of thyroid, the level here called residual level.

It has been demonstrated that iodine depletion of the thyroid increased the sensitivity of the gland to the stimulation of TSH (10). The stablization mechanism in the present study can be interpreted as being due to an increased sensitivity of the thyroid to TSH. When availability of dietary iodine is limited, the thyroidal iodine content cannot be maintained at hatching level very long and will fall to a residual level eventually. After the thyroidal iodine store drops to the residual level, the thyroid increases its sensitivity to the stimulation of TSH in some manner, thus increasing the concentrating mechanism and resulting in stabilization of the thyroid content. When availability of dietary iodine is augmented, the thyroid is able to equilibrate its iodine content at hatching level by some unknown reasons.

The stablization of the thyroidal iodine content of birds in I from 10 to 50 days of age can also be explained by the negative feedback mechanism of the pituitary-thyroid axis. When the thyroidal iodine concentration falls to the residual level, there follows a fall of circulating thyroxine level, this causes a rise of TSH in the blood and augments the activity of the gland to concentrate further the limited amount of iodine and thus stablizes its iodine store. The birds in II are exceptional, since TSH in the blood of these birds seemed not to be changed as judged by the ability to maintain constant

thyroid weights over one month, although their thyroidal iodine contents were stablized at the residual level with low circulating thyroxine levels.

Astwood and Bissell (1) reported that rapid thyroid growth toward goiter is only caused by depleted store of thyroidal iodine. In the present experiment, however, a depleted store of thyroidal iodine is shown to be not necessarily the only factor resulting in rapid thyroid growth. It is true that growth of thyroids in the birds which have the hatching level of thyroidal iodine store is slower than growth in those birds which have the residual thyroidal iodine level. However, the thyroidal iodine contents of birds in I and II at the residual level did not exhibit similar thyroid growth after attaining equilibrium. The fact that the thyroids in birds of I did show rapid growth temporarily, but the thyroids in birds of II remained fairly constant, suggests that rapid thyroid growth is caused by a factor or factors in addition to the depletion of thyroidal iodine store.

Serum Thyroxine

In general, serum thyroxine levels were high at hatching and decreased gradually as the ages of chickens increased. This observation suggests that there might be a great amount of thyroxine deposited in the yolk by the hen.

Equilibrium of serum thyroxine concentration was attained at all levels of dietary iodine, but with considerable more variation than that of the thyroidal iodine concentrations. Because the birds were killed at different times during the days (0800-2000), the diurnal

rhythm (28) might account for the wide equilibrium ranges. However, according to this previous report (28), the serum thyroxine values between 0800 and 2000 were 4.6-5.6 μg% which did not vary significantly.

It is generally considered (4,13,16) that the PBI level in birds is much lower than that in mammals. Refetoff, Robin and Fang (25) confirmed this generalization and reported further that the serum thyroxine in Leghorn chicken was 1.4 μg%. By using chromatographic techniques, however, Sadovsky and Bensandoun (28) quantitated serum thyroxine in chickens and reported that the mean concentration was 3.6-5.6 μg% which is significantly higher than that of previous reports. Thyroxine values obtained in the present study by competitive protein binding technique fell into two levels: 2-3 μg% from birds in I and II and 5-8 μg% from birds in III, IV and V. Thus the values for thyroxine in this experiment fall on either side of those obtained by Sadovsky and Bensandoun (28).

It is of interest to consider the relationship between thyroidal iodine and serum thyroxine levels after equilibrium is attained. The changes of serum thyroxine levels parallel the changes of thyroidal iodine contents at all concentrations of dietary iodine. Their correlation coefficient was +0.965 which is unusually high for a biological system.

Other Considerations

This study raises some general questions such as: (1) to what extent is the thyroid status in any one group normal or abnormal in the birds of the experiment and (2) what constitutes an adequate level of dietary iodine? Measurements of oxygen consumption or other

tests of the action of thyroid hormones were not made. Therefore, no firm conclusions can be drawn on the thyroid status of these birds from the data of this experiment. From the normal body growth rate of birds in I and II, one can speculate that the level of dietary iodine for birds of I and II is close to normal. However, taking into consideration the thyroid weight, one would eliminate I and accept II as having an adequate level of dietary iodine, since rapid thyroid growth occurred in birds of I and the thyroid growth in birds of II was nearly constant. Therefore, it is reasonable to conclude in this experiment that 5 μg iodine per 100 g of diet is an adequate level of dietary iodine for a period of 2 months. It has been documented by the American National Academy of Sciences that the dietary requirement of iodine for chickens is 35 μg/100 g of diet, however.

Another general consideration is how could the experimental design of this study be changed to improve the quality of the data? That the data were not collected at the same interval in each group of chicks is one of the weak points of the experimental design, because it is hard to compare statistically at some certain times without having data in the same period. The cages for the chicks were not uniform in regard to the size and the times of doing experiments were not identical in respect to the possible rhythm of the thyroid function augmented more or less the experimental error. In addition, the diet for one group (I & II) of chickens was not made up at one time which might have resulted in increasing experimental deviation.

The five levels of dietary iodine were determined arbitrarily prior to the beginning of the study and therefore without awareness

of the response pattern of thyroid function. It would be interesting to test the response of thyroid function to dietary iodine between 5 and 10 μg/100 g of diet, which in the present study formed a gap after the thyroid function attained an equilibrium state, particularly the thyroidal iodine concentration. Further, utilization of dietary iodine levels lower (if possible) and higher than the present studied range would be of worth particularly as concerning the production of compensatory goiter. In regard to the production of goiter, it might be a good idea to prolong the treatment of low and high iodine diet. Finally, it is unfortunate that the amount of iodine in the diets was not determined accurately with chemical analysis.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Five groups of white Leghorn cockerels were fed, from hatching untill 23-56 days of age, with different diets which varied only in iodine contents over a range of 1 μg to 1000 μg of iodine per 100 g of diet. Chicks were killed at some certain intervals sequentially. The thyroidal iodine and serum thyroxine concentrations were studied as the criteria of the thyroids response to various amounts of dietary iodine. Body weight and development of goiter were also investigated.

The concentration of iodine in the thyroid and the concentration of thyroxine in serum of chicken are capable of equilibrating in response to a wide range of dietary iodine concentration. The mechanism of adaptation, particularly for thyroidal iodine concentration, is more sensitive to change in dietary iodine between 1 and 10 than between 100 and 1000 μg/100 g diet. The thyroidal iodine contents were equilibrated early at the age of 14 days at all levels of dietary iodine. The serum thyroxine levels were equilibrated at various times depending on the amount of dietary iodine. It was concluded that birds treated with high levels of dietary iodine (higher than 10 μg/100 g of diet) equilibrated their serum thyroxine levels earlier than those treated with low levels of dietary iodine (lower than 5 μg/100 g of diet); equilibrium was attained in the former at the age of 3-20 days, while in the latter at the age of 40 days.

There was no evidence of goiter production in chickens treated with either the low-iodine diet (1 μg/100 g diet) or high-iodine diet(1000 μg/100 g diet). It was also concluded that the adaptation of chickens to low dietary iodine is by stabilization of their thyroidal iodine content with minimal enlargement of the thyroid gland. Dietary iodine higher than 100 μg/100 gm diet depressed body growth of chickens significantly. A good correlation (coefficient = + 0.965) was found between thyroidal iodine content and serum thyroxine level in the chickens.

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APPENDIX

TABLE III

BODY AND THYROID (ONE GLAND) WEIGHTS; IODINE CONCENTRATIONS IN THYROID AND SERUM THYROXINE LEVELS IN GROUP I

Hatching day (*)

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	36.7	3.80	0.568	6.43
2	39.4	3.73	1.458	14.43
3	37.8	2.40	1.216	6.17
4	41.7	4.30	0.781	7.67
5	41.7	2.60	1.200	7.31
6	42.7	3.45	0.916	9.55
7	34.3	2.50	1.040	9.80
8	41.7	2.80	0.600	4.65
9	34.8	2.65	1.313	5.72
10	36.7	3.40	1.224	10.35
MEAN ± SE	38.75 ± 0.98	3.16 ± 0.21	1.032 ± 0.097	8.21 ± 0.91
2-day age (*)				
1	45.7	3.29	0.632	8.83
2	41	2.50	1.200	6.26
3	42.3	3.01	1.090	6.79
4	41.4	3.75	1.877	7.75
5	42.9	4.10	0.702	3.64
6	40.5	3.45	0.626	5.05
7	43.3	3.50	1.006	5.61
8	43.6	3.17	1.388	4.64
9	41.7	2.20	2.173	10.77
10	39	2.78	0.691	4.82
MEAN ± SE	42.14 ± 0.59	3.18 ± 0.18	1.139 ± 0.170	6.42 ± 0.69

* Thyroid weights of 2 glands.

TABLE III—Continued

4-day age (*)

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	48.0	2.65	0.927	6.99
2	50.0	3.20	1.065	14.14
3	45.5	2.05	0.363	7.21
4	48.1	2.35	0.511	7.55
5	56.5	5.80	0.303	10.59
6	51.4	4.35	—	10.85
7	50.4	2.85	0.407	12.09
8	44.6	1.85	1.557	10.81
9	48.2	4.05	0.514	10.77
10	40.7	2.40	0.680	7.26
MEAN ± SE	48.34 ± 1.35	3.16 ± 0.39	0.703 ± 0.137	9.83 ± 0.78

7-day age (*)

1	85.0	2.00	0.606	4.07
2	79.2	1.66	0.355	7.28
3	65.2	2.14	0.374	6.02
4	65.8	2.29	0.227	6.43
5	70.9	2.51	0.239	4.34
6	89.6	2.23	0.215	6.07
7	74.0	2.82	1.113	3.88
8	70.0	2.70	0.237	4.83
9	88.6	2.96	0.270	8.46
10	57.6	1.87	0.861	2.83
MEAN ± SE	74.59 ± 3.40	2.32 ± 0.13	0.450 ± 0.099	5.42 ± 0.55

*Thyroid weights of 2 glands.

10-day age

1	79.8	1.95	0.219	3.96
2	98.0	2.50	0.124	8.84
3	70.7	1.65	0.205	5.11
4	96.7	2.65	0.098	4.98
5	92.3	1.85	0.238	3.19
6	117.3	3.65	0.921	3.28
7	55.2	1.85	0.043	5.58
8	109.6	2.65	0.036	4.64
9	94.5	1.75	0.426	5.59
10	90.8	2.90	0.128	3.68
MEAN ± SE	90.42 ± 5.76	2.34 ± 0.20	0.244 ± 0.083	4.89 ± 0.52

TABLE III—Continued

Bird No	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
14-day age				
1	144.9	3.81	0.394	4.96
2	127.7	4.50	0.067	2.55
3	128.5	2.83	0.106	3.11
4	150.1	4.53	0.068	8.62
5	139.3	3.20	0.075	3.38
6	138.7	3.58	0.105	4.79
7	133.7	3.18	0.428	3.76
8	141.0	4.76	0.185	6.99
9	113.6	1.60	0.338	8.60
10	137.7	3.98	0.070	5.41
MEAN ± SE	135.49 ± 3.26	3.60 ± 0.30	0.184 ± 0.046	5.22 ± 0.70
17-day age				
1	134.0	4.25	0.047	6.08
2	151.1	3.85	0.088	5.38
3	161.9	5.10	0.038	5.85
4	167.8	4.55	0.110	7.09
5	172.2	8.90	0.056	5.03
6	141.7	3.55	0.081	2.22
7	167.2	3.60	0.077	4.40
8	169.7	5.25	0.045	4.16
9	160.7	4.30	0.276	3.15
10	156.8	4.25	0.179	4.91
MEAN ± SE	158.31 ± 3.99	4.76 ± 0.49	0.100 ± 0.024	4.83 ± 0.45
21-day age				
1	242.3	7.25	0.033	5.60
2	210.5	9.00	0.009	6.03
3	194.4	5.90	0.078	5.09
4	197.8	6.10	0.067	5.63
5	208.8	6.10	0.014	3.61
6	219.6	6.70	0.030	2.56
7	225.3	6.05	0.036	5.01
8	224.2	7.20	0.031	4.06
9	250.5	11.55	0.037	3.81
10	227.0	5.85	0.068	4.43
MEAN ± SE	220.04 ± 5.67	7.17 ± 0.57	0.040 ± 0.007	4.58 ± 0.34

TABLE III—Continued

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
29-day age				
1	353.2	15.35	0.116	6.49
2	332.8	12.35	0.081	2.35
3	297.5	14.25	0.086	4.19
4	351.3	14.65	0.077	2.99
5	339.0	13.05	0.098	2.94
6	394.2	25.55	0.065	2.51
7	331.1	20.25	0.053	6.28
8	363.4	21.05	0.083	6.52
9	305.4	12.25	0.029	4.51
10	320.1	10.35	0.078	5.28
MEAN ± SE	338.80 ± 9.02	15.91 ± 1.52	0.077 ± 0.008	4.41 ± 0.53
43-day age				
1	525	17.10	0.088	3.57
2	521	15.40	—	3.39
3	502	19.75	0.077	2.10
4	484	24.95	0.071	3.60
5	496	24.45	0.170	2.11
6	484	21.50	0.091	2.59
7	536	31.25	0.129	2.39
8	395	4.35	0.054	1.41
9	439	10.90	0.081	1.41
10	477	16.75	0.065	1.28
MEAN ± SE	485.9 ± 13.42	18.64 ± 2.41	0.091 ± 0.011	2.39 ± 0.28
56-day age				
1	508	13.20	0.163	3.44
2	696	12.90	0.207	2.96
3	490	12.10	0.096	3.62
4	515	22.15	0.408	2.45
5	362	22.40	0.245	2.63
6	756	32.10	0.308	2.23
7	687	16.10	0.311	2.22
8	493	9.10	0.154	1.97
9	560	14.60	0.414	2.28
10	617	16.60	0.140	2.99
MEAN ± SE	568.4 ± 37.87	17.13 ± 2.13	0.245 ± 0.035	2.78 ± 0.20

TABLE IV

BODY AND THYROID (ONE GLAND) WEIGHTS;
IODINE CONCENTRATIONS IN THYROID AND
SERUM THYROXINE LEVELS IN GROUP II

2-day age (*)

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	35.5	4.05	3.022	11.15
2	36.3	2.72	0.912	12.10
3	43.6	3.00	0.480	6.22
4	44.1	4.12	2.913	9.59
5	43.5	3.54	2.825	9.32
6	52.6	2.51	1.004	6.45
7	41	2.95	1.410	13.53
8	40.5	3.14	1.529	9.71
9	35.8	3.61	2.260	2.66
10	42.6	2.25	3.698	9.21
MEAN±SE	41.55±1.62	3.19±0.20	2.005±0.343	8.99±1.00

4-day age (*)

1	47.0	2.15	1.735	11.37
2	47.8	3.00	2.360	14.25
3	38.6	2.80	3.057	6.35
4	54.9	4.65	—	5.45
5	50.9	2.50	1.232	8.81
6	40.8	2.45	1.502	13.66
7	38.0	3.50	2.731	8.13
8	55.9	2.85	1.095	8.90
9	44.9	2.60	1.200	8.44
10	45.3	3.15	0.381	12.00
MEAN±SE	46.41±1.98	2.97±0.22	1.699±0.288	9.74±0.94

* Thyroid weights of 2 glands.

TABLE IV—Continued

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
7-day age				
1	61.1	1.95	0.251	6.81
2	73.2	2.73	1.070	8.21
3	71.6	2.75	1.716	4.71
4	77.5	2.74	1.080	3.80
5	78.3	2.35	0.766	5.78
6	64.4	3.00	0.130	7.19
7	73.8	1.45	0.517	7.04
8	68.4	2.07	0.628	6.67
9	73.4	1.93	0.472	8.47
10	67.3	1.55	0.639	5.78
MEAN ± SE	70.9 ± 1.75	2.25 ± 0.17	0.727 ± 0.147	6.45 ± 0.46
10-day age				
1	76.2	1.25	0.584	5.99
2	86.5	1.15	0.452	2.71
3	72.0	1.75	0.634	2.20
4	70.4	1.80	0.544	1.60
5	78.6	1.55	0.181	6.37
6	84.6	1.95	0.395	5.62
7	99.5	3.05	0.367	5.09
8	68.9	1.75	0.463	1.74
9	93.1	2.05	1.058	6.05
10	97.0	3.30	0.370	1.42
MEAN ± SE	82.68 ± 3.54	1.96 ± 0.22	0.505 ± 0.074	3.88 ± 0.66
14-day age				
1	136.2	5.05	0.032	5.23
2	141.0	3.60	0.119	3.01
3	118.7	6.06	0.139	3.42
4	100.5	1.16	0.198	2.52
5	116.7	2.65	0.181	3.66
6	124.3	3.31	0.266	6.47
7	111.6	2.91	0.110	3.58
8	136.0	3.70	0.168	4.89
9	120.3	3.74	0.061	7.15
10	92.0	2.43	0.037	4.98
MEAN ± SE	119.73 ± 4.98	3.46 ± 0.43	0.131 ± 0.024	4.49 ± 0.48

TABLE IV—Continued

17-day age

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	157.1	5.10	0.066	2.35
2	170.6	7.70	0.035	2.24
3	146.8	3.90	0.103	2.23
4	146.6	2.50	0.093	0.61
5	112.1	4.30	0.054	0.67
6	153.4	5.80	0.058	1.03
7	176.7	5.20	0.038	1.92
8	152.2	3.05	0.234	2.11
9	139.0	3.65	0.126	1.20
10	140.3	4.15	0.060	1.34
MEAN±SE	149.48±5.66	4.54±0.47	0.087±0.019	1.59±0.22

21-day age

1	191.3	2.00	0.390	3.32
2	174.8	5.25	0.079	2.27
3	167.7	3.55	0.096	2.65
4	132.7	7.05	0.086	2.41
5	248.2	6.60	0.091	2.41
6	215.6	8.10	0.075	2.37
7	200.5	6.50	0.059	3.77
8	195.4	7.40	0.128	3.16
9	197.5	7.85	0.057	2.92
10	162.3	6.20	0.056	3.38
MEAN±SE	188.6±10.01	6.05±0.62	0.112±0.032	2.87±0.16

30-day age

1	241.0	7.20	0.068	3.23
2	251.3	4.80	0.118	3.90
3	286.3	7.50	0.113	4.90
4	273.0	8.70	0.145	3.42
5	349.2	10.50	0.059	6.54
6	214.0	6.20	0.054	3.80
7	238.5	6.95	0.068	2.16
8	252.7	9.85	0.061	4.51
9	276.2	15.00	0.074	3.14
10	217.3	3.90	0.255	1.66
MEAN ± SE	259.95±12.47	8.06±1.01	0.101±0.020	3.73±0.44

TABLE IV—Continued

38-day age

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	382.7	6.30	0.189	3.00
2	381.9	9.05	0.075	4.19
3	411.7	9.95	0.079	3.59
4	298.2	3.85	0.114	2.18
5	361.3	10.25	0.088	3.83
6	452.0	13.65	0.082	3.75
7	394.6	14.85	0.155	3.34
8	350.0	9.55	0.044	3.39
9	347.4	8.20	0.024	2.60
10	254.4	4.70	0.353	1.07
MEAN±SE	363.37±17.78	9.04±1.11	0.140±0.025	3.09±0.29

52-day age

1	488	18.85	0.049	2.29
2	563	12.85	0.109	1.90
3	615	18.55	0.115	1.78
4	508	11.60	0.160	2.60
5	620	11.05	0.084	2.11
6	560	17.35	0.055	2.26
7	388	3.50	0.144	1.88
8	608	20.15	0.148	2.54
9	715	16.80	0.054	1.32
10	588	12.65	0.068	1.77
MEAN±SE	565.3±28.10	14.54±1.45	0.098±0.013	2.05±0.12

TABLE V

BODY AND THYROID (ONE GLAND) WEIGHTS:
IODINE CONCENTRATIONS IN THYROID AND
SERUM THYROXINE LEVELS IN GROUP III

3-day age (*)

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	51.2	1.50	1.109	7.24
2	50.2	1.35	1.484	3.39
3	46.9	1.15	1.602	4.73
4	46.1	1.55	1.058	10.25
5	48.1	1.35	1.535	4.37
6	45.6	1.45	0.767	6.84
7	51.8	1.15	1.110	8.36
8	40.2	1.55	0.945	5.00
9	52.4	1.75	0.343	3.08
10	45.1	1.70	0.729	2.92
MEAN ± SE	47.76 ± 1.19	1.45 ± 0.06	1.068 ± 0.126	5.62 ± 0.78
7-day age				
1	55.4	1.75	0.480	5.35
2	50.6	1.75	0.903	4.58
3	65.0	1.30	0.246	7.77
4	65.4	2.10	0.512	5.42
5	70.7	1.60	0.540	6.48
6	62.4	1.60	0.675	5.13
7	56.4	1.20	0.507	4.03
8	66.5	1.90	0.872	6.48
9	45.0	2.15	0.539	3.21
10	77.1	2.50	0.156	3.83
MEAN ± SE	61.45 ± 3.05	1.79 ± 0.13	0.543 ± 0.075	5.23 ± 0.44

* Thyroid weights of 2 glands.

TABLE V—Continued

18-day age

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	123.1	2.55	0.901	6.76
2	143.0	3.40	0.629	7.18
3	119.6	2.45	0.898	6.90
4	143.6	3.40	0.744	6.14
5	136.8	3.65	1.732	4.45
6	120.4	5.25	2.118	8.42
7	138.8	4.65	0.508	5.38
8	135.3	3.50	0.740	4.96
9	153.5	3.85	1.704	5.26
10	161.7	4.95	1.325	5.76
MEAN ± SE	137.58 ± 4.40	3.77 ± 0.30	1.130 ± 0.175	5.92 ± 0.43

23-day age

1	172.4	3.45	0.916	8.47
2	151.1	3.50	0.617	6.12
3	160.9	3.80	0.532	7.66
4	144.9	2.35	1.680	5.03
5	175.3	3.05	1.698	4.96
6	189.4	3.65	1.150	3.70
7	133.4	2.75	2.002	4.68
8	180.8	2.65	0.891	6.66
9	158.2	3.40	1.235	7.19
10	158.9	2.95	1.801	6.19
MEAN ± SE	162.53 ± 5.42	3.16 ± 0.15	1.252 ± 0.164	6.07 ± 0.47

TABLE VI

BODY AND THYROID (ONE GLAND) WEIGHTS: IODINE CONCENTRATIONS IN THYROID AND SERUM THYROXINE LEVELS IN GROUP IV

4-day age(*)

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	52.2	5.45	1.339	12.37
2	43.1	2.80	1.000	7.23
3	44.2	2.10	1.124	12.09
4	47.7	3.55	1.409	12.82
5	47.3	2.90	1.335	8.90
6	46.6	2.85	1.359	12.07
7	45.9	2.60	1.292	9.70
8	47.5	3.30	1.430	9.18
9	46.1	2.05	1.112	10.09
10	42.8	2.50	1.018	10.21
MEAN ± SE	46.34 ± 0.86	3.01 ± 0.31	1.242 ± 0.051	10.47 ± 0.58

8-day age (*)

1	46.2	2.45	1.414	13.15
2	52.5	3.35	1.056	8.87
3	51.1	2.85	1.656	6.12
4	51.4	2.25	1.547	8.78
5	50.6	2.84	1.452	5.36
6	67.3	3.80	0.881	9.83
7	53.3	2.30	1.017	14.17
8	49.8	2.55	1.153	7.29
9	60.4	3.15	1.537	8.15
10	61.6	2.50	1.326	9.41
MEAN ± SE	54.42 ± 2.06	2.80 ± 0.16	1.304 ± 0.083	9.11 ± 0.88

* Thyroid weights of 2 glands.

TABLE VI—Continued

17-day age

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	53.0	1.40	0.758	5.31
2	85.0	2.25	1.979	10.11
3	77.6	1.45	0.698	10.39
4	75.4	1.65	0.878	6.27
5	75.0	2.50	1.571	3.15
6	77.1	2.55	0.966	8.62
7	91.7	1.85	0.825	6.30
8	82.8	1.40	0.711	6.81
9	75.2	1.35	0.313	5.33
10	77.1	1.20	0.812	9.07
MEAN ± SE	76.99 ± 3.16	1.76 ± 0.16	0.951 ± 0.151	7.14 ± 0.74
24-day age				
1	109.5	1.65	0.363	3.54
2	92.5	1.70	1.306	2.74
3	134.5	2.55	1.597	4.11
4	83.3	2.55	1.440	5.54
5	92.7	2.45	0.759	3.75
6	124.3	2.45	1.685	4.20
7	76.2	2.65	1.063	4.06
8	83.8	1.65	0.304	4.31
9	97.3	2.05	0.628	4.19
10	107.1	2.40	1.603	2.79
MEAN ± SE	100.12 ± 5.92	2.21 ± 0.13	1.075 ± 0.167	3.92 ± 0.25
31-day age				
1	86.7	1.50	0.537	4.10
2	105.0	3.60	1.137	5.10
3	89.4	1.55	0.590	6.05
4	96.1	2.80	0.943	6.34
5	64.5	1.30	0.514	4.37
6	114.6	1.30	0.584	8.27
7	82.4	1.10	0.473	5.91
8	86.3	1.50	0.348	5.82
9	76.7	2.80	1.228	4.60
10	67.4	0.90	2.026	5.55
MEAN ± SE	86.91 ± 4.94	1.84 ± 0.28	0.838 ± 0.162	5.61 ± 0.38

TABLE VII

BODY AND THYROID (ONE GLAND) WEIGHT: IODINE CONCENTRATIONS IN THYROID AND SERUM THYROXINE LEVELS IN GROUP V

4-day age (*)

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
1	43.5	3.35	5.158	3.99
2	44.9	4.28	5.346	5.25
3	44.4	3.37	4.871	5.21
4	52.5	5.85	5.238	4.20
5	43.2	3.07	5.509	6.67
6	37.7	2.55	2.212	5.10
7	40.9	3.31	5.955	5.67
8	39.7	2.26	2.292	4.17
9	39.7	2.87	2.202	4.43
10	46.6	2.91	2.034	5.72
MEAN ± SE	43.31 ± 1.34	3.38 ± 0.32	4.082 ± 0.524	5.04 ± 0.27
9-day age				
1	51.5	1.85	0.935	5.53
2	48.2	1.60	0.554	2.78
3	48.1	1.35	0.948	6.00
4	61.4	2.10	1.867	8.34
5	51.0	1.60	0.516	4.64
6	48.3	1.65	1.158	2.91
7	61.3	2.45	2.351	7.09
8	58.6	1.10	0.582	8.32
9	49.7	2.00	0.960	7.45
10	60.8	2.50	1.587	6.05
MEAN ± SE	53.85 ± 1.86	1.82 ± 0.14	1.146 ± 0.193	5.91 ± 0.63

* Thyroid weights of 2 glands.

TABLE VII—Continued

Bird No.	Body Wt. (g)	Thyroid Wt. (mg)	Iodine Conc. in Thyroid (μg/mg)	Serum T_4 (μg%)
15-day age				
1	75.7	1.80	0.118	4.48
2	84.5	1.50	1.167	8.71
3	67.2	2.10	1.790	6.39
4	70.8	1.95	0.938	6.36
5	64.5	1.90	0.963	4.01
6	73.1	1.70	0.759	7.10
7	64.8	1.15	0.637	5.97
8	63.5	1.80	0.654	4.75
9	59.3	1.60	2.714	5.05
10	64.1	2.10	0.964	5.46
MEAN ± SE	68.75 ± 2.34	1.86 ± 0.12	1.072 ± 0.227	5.83 ± 0.44
23-day age				
1	82.7	1.70	0.475	6.36
2	94.3	2.20	4.018	4.78
3	94.2	2.55	1.650	6.81
4	89.3	1.10	0.751	3.85
5	81.8	1.95	1.210	4.57
6	104.0	1.90	0.589	4.75
7	104.1	1.25	0.376	2.50
8	100.7	2.15	1.258	6.39
9	74.2	1.35	0.590	3.43
10	84.0	1.25	0.645	4.24
MEAN ± SE	90.93 ± 3.24	1.74 ± 0.15	1.156 ± 0.343	4.77 ± 0.44
31-day age				
1	81.0	2.00	1.645	8.00
2	95.6	1.90	0.540	8.30
3	117.9	3.60	2.053	7.70
4	100.5	3.00	1.083	6.16
5	65.4	0.70	0.726	10.13
6	100.2	2.25	1.004	9.37
7	91.9	1.80	0.848	5.89
8	114.8	1.65	0.909	4.75
9	110.2	2.05	1.500	6.17
10	100.1	1.60	0.798	3.20
MEAN ± SE	97.76 ± 4.99	2.06 ± 0.25	1.111 ± 0.150	6.97 ± 0.67

TABLE VIII

THYROID WEIGHTS EXPRESSED IN MG OF ONE GLAND PER 100 G BODY WEIGHT OF FIVE GROUPS OF CHICKS, MEAN OF TEN CHICKS WAS GIVEN IN EACH FIGURE

Age (day)	Thyroid Wt., (mg/100 g body wt.)				
	Group I	Group II	Group III	Group IV	Group V
Hatching	4.08*				
2	3.78*	3.84*	—	—	—
3	—	—	1.52*	—	—
4	3.27*	3.42*	—	3.25*	3.90*
7	1.56*	3.17	2.91	—	—
8	—	—	—	2.58*	—
9	—	—	—	—	3.38
10	2.59	2.37	—	—	—
14	2.66	2.89	—	—	—
15	—	—	—	—	2.71
17	3.01	3.04	—	2.29	—
18	—	—	2.74	—	—
21	3.26	3.21	—	—	—
23	—	—	1.94	—	1.91
24	—	—	—	2.21	—
29	4.70	—	—	—	—
30	—	3.10		—	—
31	—	—		2.12	2.11
38	—	2.49		—	—
43	3.84	—			
52	—	2.57			
56	3.01	—			

* mg of 2 thyroids per 100 g body weitht divided by 2.

TABLE IX

RECOVERY OF T_4

Tube #	% Recovery of ^{125}I-T_4	% Recovery of Stable T_4
1	97.75	97.9
2	103.73	89.3
3	102.85	106.9
4	106.48	97.2
5	105.91	103.0
6	99.93	101.3
7	99.51	96.2
8	96.36	106.0
9	103.76	100.4
10	101.83	98.2
11	102.03	92.9
12	100.67	109.4
MEAN $\pm$ SE	101.62 ± 0.98	99.89 ± 1.85

VITA

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