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THE EFFECT OF EXOGENOUS
THYROXINE ON THE
IODINE TURNOVER OF THE
CHICK THYROID GLAND

Thesis for the Degree of M. S.
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Robert L. Cornwall
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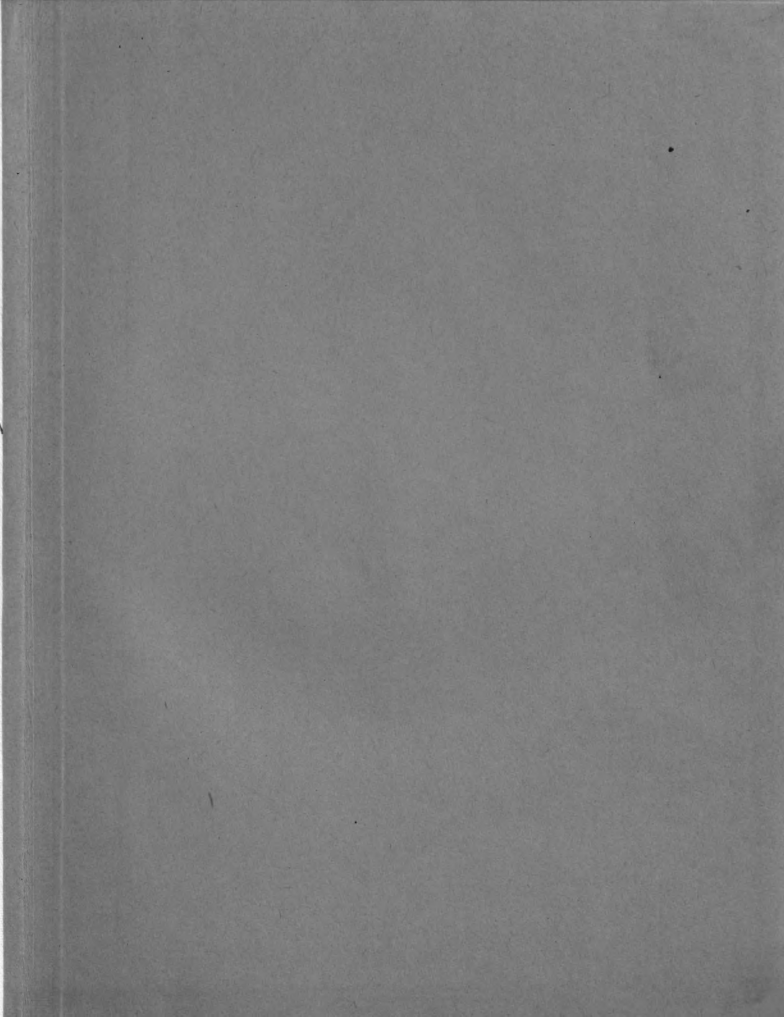
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E. P. Keimke
Major professor

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THE EFFECT OF EXOGENOUS THYROXINE ON THE IODINE TURNOVER
OF THE CHICK THYROID GLAND

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Robert L. Cornwall

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INTRODUCTION

Since Baumann's discovery of relatively high concentrations of iodine in the thyroid gland in 1895, many species have been studied to determine the significance of its presence in the gland. From preliminary work, many questions have arisen concerning the mechanism whereby the thyroid collects and utilizes ingested iodine in the manufacture of its secretion and the influence of the thyroid hormone on the efficiency and well being of the organism.

Important among the techniques that have evolved for the study of thyroid physiology is that employing the unique and useful radio-active isotope of iodine (I^{131}). With this material, newly formed molecules of organically bound iodine can be tagged, thus facilitating determinations relative to the rate at which the thyroid gland is manufacturing and discharging its product. Coupled with methods of longer standing, radio-iodine provides the student of thyroid physiology with an adequate armamentarium with which to attack the problem of his choosing.

The interrelationship of the thyroid and pituitary glands has long been recognized. Numerous reports deal with the necessity of the anterior pituitary substance, thyrotropin, for the maintenance of the thyroid's functional integrity. On the other hand, it is well established that administered thyroïdal substances suppress the output of thyroid stimulating hormone from the pituitary. It is the purpose of this study to demonstrate the influence of rela-

tively small, daily amounts of exogenous thyroxine on the collection and turnover rates of tracer doses of radio-active iodine in the chick thyroid gland.

REVIEW OF LITERATURE

Thyroid Activity and Iodine Content

Shortly following Baumann's discovery of iodine in the human thyroid, many workers became concerned with the nature of the thyroid hormone. It was soon learned that a large percentage of the iodine found in the gland was organically bound. In 1898 Ostwald first described the contents of the follicular lumina as a protein substance which he chose to call thyroglobulin. Kendall (1915) was successful in isolating a crystalline material from thyroid tissue which contained over 60% iodine by weight. He named this substance thyroxine. But it was not until 1926 that the constitution of the compound was established by Harington and another year before he and Barger synthesized thyroxine. The synthetic material contained about 65% iodine on a molecular weight basis.

The establishment of the nature of the thyroid's secretion initiated a great number of questions relative to the mechanisms involved in the collection and conversion of iodine to the hormonal substance present in the gland. Harington and Barger (1927) proposed the theory that tyrosine, found to be present in the gland, was iodinated to form diiodotyrosine and a subsequent coupling of two of the molecules, with the loss of one side chain, resulted in a molecule of thyroxine. The entire process has yet to be demonstrated but all indications substantiate this original theory. Recent studies have been interpreted by Astwood (1949) to indicate that the steps

involved in the formation of thyroxine are: 1) the concentration of iodide by the thyroid; 2) the oxidative conversion of iodide to the organic form, presumably in combination on the diiodotyrosyl radical of a protein; and 3) the oxidative coupling of diiodotyrosyl radicals to form thyroxyl groups. He has shown that the first of these steps is inhibited by thiocyanate while the second may be altered through the action of thiocarbonamide and amino benzene type compounds, probably by inhibiting an enzyme system responsible for this oxidative process. The sequence of manufacture of thyroid hormone has been studied in tissue slices of the rat thyroid by Leblond and Gross (1949). They used the radio-autograph method to determine the distribution of a tracer dose of radio-active iodine (I^{131}) in the various areas of the gland as a function of time following the injection of the isotope. They find that the I^{131} is found chiefly in the apical portion of the cells and periphery of the lumen one hour after its administration. After 24 hours the activity is evenly distributed in the colloid of the follicular lumina. This shows that the conversion of iodine to the organic state has gotten well under way in the short period of one hour and is complete after 24 hours. Indeed, Chaikoff and Taurog (1949) find that 95% of the tracer dose of I^{131} that is found in the thyroid is in the organic state as early as 15 minutes and of this 80% is present as diiodotyrosine and 10-15% as thyroxine while 5% remained unbound.

The total iodine concentration in the thyroid is several

hundred times that in the blood plasma. The mechanisms involved in the collection of relatively vast amounts of iodide and conversion to organic iodine are not fully explained. The distribution of the thyroid's total iodine, however, has been quite extensively investigated. Lein (1943), by fractionation of rabbit thyroid tissue, found the inorganic iodine concentration to be negligible about 12 hours after the intravenous administration of 35 mg. of iodine labeled with I^{131} . The protein-bound iodine was increasing at the end of the 12 hour observation period. The same was reported by Perlman, Chaikoff and Morton (1942) on their studies of the rat. The accumulation of labeled iodine was largely in the organically-bound fraction. Chaikoff and Taurog (1949) determined values for both I^{131} and stable iodine (I^{127}) distribution in organic combination in the rat. About 70% of the stable iodine is found in the diiodotyrosine fraction while 27% is incorporated into thyroxine with 3 - 4% remaining in the inorganic state. The I^{131} reached its peak concentration about 14 hours after administration. At this time about 73% of the collected isotope was in the diiodotyrosine form and 25% in the thyroxine fraction. These values remained relatively constant up to 50 hours, when the procedure was terminated. These data are in close agreement with other authors reporting on rat glands - (Taurog and Chaikoff, 1947; Morton, et al., 1942; Keston, et al., 1944). In the dog, Mann, Leblond and Warren (1942) were not able to show that inorganic iodine exists in the thyroid at all. Using I^{131} as

an indicator, they state, "The inorganic iodine level does not rise high enough in one half to 48 hours following the injection to indicate it as a source of iodine for organic incorporation into diiodotyrosine and thyroxine". It was suggested that the conversion must take place at the level of the membrane.

The above findings, then, indicate that the conversion of iodide to the organically-bound form in the thyroid is a surprisingly rapid event and that little, if any, iodide is stored in the gland at any particular time.

Among the factors influencing the amount of iodine concentrated in the thyroid is the relative intake and blood level of iodide. Taurog and Chaikoff (1946) have shown that the amounts of thyroxine and total iodine in the normal rats' gland increases as the daily iodine intake increases. They were able to demonstrate a relative increase in animals receiving 1, 2 and 78 gamma per day but no increase above the latter value. The total storage capacity was 130-140 mg. % of total iodine, 40 - 50 mg. % being found as thyroxine iodine. The post-absorptive value for protein-bound iodine in the plasma was proportional to the iodine intake as well. Again, there was no increase in animals receiving more than 78 gamma of iodine daily. It was seen that the plasma protein-bound iodine level was dependent on the thyroxine content of the thyroid and limited by the capacity of the gland to produce thyroxine. Fulton, in his Textbook of Physiology (1949), states that the normal human thyroid contains about

50 mg. % total iodine, about 1 mg. % as inorganic iodide, while the plasma level is expressed as micrograms percent. Wolff and Chaikoff (1948) report that plasma inorganic iodine concentrations above 30 gamma/100 ml. tend to inhibit thyroid function completely. They also find (1948a) that excessive iodine interferes with the iodination of tyrosine and the subsequent formation of thyroxine although no thyroid hypertrophy results from the inhibition of thyroid activity.

There exists a relationship between the weight of the thyroid and its iodine content. Marine (1935) concluded that the iodine content was inversely proportional to the gland's weight in human subjects. However, his studies were carried out under conditions of simple or endemic goiter where hyperplasia of the gland is the result of insufficient dietary iodine. When iodine was supplied in the diet the glands of these subjects assumed the normal size and the weight-iodine content relationship should have become more direct. Cruikshank (1929) has determined the thyroid iodine content of chicks. While noting the changes occurring in iodine content of normal chicks at various seasons of the year, a weight change in the glands was found and was proportional to the iodine content. Both factors increased during winter months. Since Reineke and Turner (1945) find an increased secretion rate of chick thyroids during winter months, it would seem that not only is the gland weight of normal chicks indicative of activity but also the iodine content. In the review by Schultz and Turner (1945), it is likewise stated that a direct

proportionality exists between gland weight and iodine content in chicks deprived of pituitary thyroid-stimulating hormone. The report on accumulation of colloid in the atrophic glands which increases both weight and iodine content and the relationship remains a proportional one. It is interesting to note here that the existing parallelism of gland weight, iodine content and secretory activity of the normal chick thyroid leads one to believe that this animal's gland must store limited quantities of colloid in contrast to the mammals.

The formation of thyroxine is not limited to the thyroid gland alone. Reineke (1949) describes methods for the direct iodination of casein which yields about 3% active thyroidal material. He states further that the active component of the protein has been demonstrated to be l-thyroxine, the active substance produced by the thyroid (Harrington and Salter, 1930), and the form shown to be about twice as active as the racemic mixture by Reineke and Turner (1945). Dvoskin (1947) presents evidence that a thyroxine-like material is actually formed at the injection site of iodine (in oil) and slowly diffuses into the body. It has also been postulated that minute amounts of thyroxine are formed in the completely thyroidectomized animal and can be found in circulating plasma (Chaikoff, Taurog and Reinhardt, 1947).

A number of drugs are known that inhibit the various phases of thyroxine formation in the gland. Through the use of these compounds, the actual processes whereby the thyroid

hormone is produced have become clearer. Astwood (1949) classifies the substances which, in one way or another, depress the function of the thyroid under four headings: 1) Thyroid hormone; 2) Iodine; 3) Thiocynate ion; 4) Antithyroid substances proper (compounds which interfere with thyroid hormone synthesis). The first of these will be dealt with later and the second has already been mentioned. Regarding the thiocynate ion, Wolff, Chaikoff, Taurog and Rubin (1946), as well as Vanderlaan and Bissel (1946), have shown that this substance markedly inhibits the accumulation of iodine by the thyroid gland. Vanderlaan and Vanderlaan (1947) have further shown that, when as little as 1 mg. of potassium thiocynate is administered to animals whose thyroids contain a large quantity of inorganic iodine, there is an immediate discharge of all of it from the thyroid gland. Because the concentration of thiocynate is less in thyroid tissue than in the blood of treated animals, Astwood (1949) eliminates the possibility of competitive absorption of this ion by the thyroid gland, thus displacing the iodine ions. It is known that thiocynate is not goitrogenic when large amounts of iodide are added to the diet but this is probably a factor of simple diffusion of iodide from the blood into the thyroid gland rather than any inhibition of the inherent goitrogenic properties of thiocynate.

Compounds which interfere with thyroid hormone synthesis are many in number.. Those inhibiting the oxidative conversion of iodide to the organic form are believed to be the

thiocarbonamide (thiourea and thiouracil) and amino benzene type compounds (the sulfonamides), as stated by Astwood (1949). Several workers have reported the effects of thiourea and thiouracil on thyroid gland function. (Larson, et al, 1945; Mixner, Reineke and Turner, 1944; Reineke, Schultz and Turner, 1944; Keston, et al, 1944; Astwood, 1943; Griesback, Kennedy and Purves, 1941; and many others). It is generally agreed that these drugs cause hypertrophy of the thyroid gland, decrease the total iodine content of the gland, but do not inhibit the uptake and turnover of inorganic iodine as with the thiocynate ion. Less iodine is found in the gland, presumably because of a reduced rate of synthesis of iodine-containing compounds. This is well pointed out in studies concerning the uptake and distribution of I^{131} between inorganic and organic fractions. Astwood and Bissel (1944), however, have shown that considerable radio-iodine is rapidly taken up by rat thyroids which have been depleted of iodine by thiouracil treatment but does not enter into organic combination. In contrast, Larson, et al, (1945), find little I^{131} collected in the chick thyroid made hyperplastic by thiouracil and again suggest inhibition of organic iodine formation.

Thyroid - Pituitary Interrelationship

Both the structure and functional capacity of the thyroid gland is under delicate regulatory influences which insure that the quantity of hormone secreted is appropriate for the requirements of the body. Foster and Smith (1926) were

among the first to note that the basal metabolic rate was below normal in hypophysectomized rats and that pituitary implants resulted in a return to the normal rate. It was further found, by Loeb and Basset (1929), that certain hormones of the anterior pituitary caused marked hypertrophy of the thyroid glands of normal guinea pigs. An increase in acinar cell height and decreased amounts of follicular colloid were described by these authors. So it was established that the pituitary gland was necessary for normal function of the thyroid and that an excess of the pituitary hormone would cause an increase in the activity of the thyroid gland. It has also been well shown that the administration of excessive thyroidal material will reduce the size and so the functional activity of the normal thyroid (Reforzo - Membrives, 1943; Parker, 1943; Irwin, Reineke and Turner, 1943; Koger and Turner, 1943; and others).

With the appearance of the goitrogenic compounds and a method of inhibiting the formation of thyroid hormone it was possible to relate the degree of thyroid hormone synthesis and its secretion into the circulation to the relative size, histologic appearance and colloid storage of the thyroid gland. A method for determining the secretion rate of the gland has been based on the amount of exogenous thyroxine necessary to prevent thyroid hypertrophy in thiouracil-treated chicks (Reineke, Mixner and Turner, 1945; Schultz and Turner, 1945), and rats (Dempsey and Astwood, 1943). It is assumed that thyroid hypertrophy is the result of inhibition of thyroid hor-

mone synthesis and increased release of thyrotropic hormone and that administered thyroxine suppresses the latter event (Astwood, et al., 1943; Rawson, et al., 1944; Griesback, Kennedy and Purves, 1941; and others). Larson, Keating, Peacock and Rawson (1945) have reported that the increase of mean acinar cell height following thiouracil treatment was similar to that expected with the injection of thyrotropic hormone. That the goitrogenic activity of these drugs is mediated by way of the pituitary has been shown by the fact that hypophysectomy prevents the occurrence of the changes described (Astwood, et al., 1943).

On considering the capacity of the thyroid gland to collect and store iodine and the influence of pituitary secretion on this phenomenon, Astwood and Bissel (1944) report that the thyroids of rats treated with thyroid-stimulating hormone contained almost the same amount of iodine as the controls. A combination of thiouracil and thyrotropin, however, allows the thyroid to collect more iodine than is found with thiouracil treatment alone. This suggests that thyrotropin may aid in the deposition of iodine, although the thyroid made hyperplastic with thyrotropin is not further increased in size by thiouracil administration. In some of the earlier studies with radio-active iodine, Hertz and Roberts (1941) found an increase in thyroid size, iodine collecting capacity and acinar cell height in rabbits given thyroid-stimulating hormone. The same finding was reported by Leblond and Sue (1941) in their work with the guinea pig. Salter

(1940) states that the iodine content may be depleted to one-tenth that of normal when thyrotropin is administered and presumes that the thyroxine is removed as rapidly as it forms, thus explaining the phenomenon. Keating, Rawson, Peacock and Evans (1945) point out the factor of time and its relation to the effect of thyrotropin on iodine collection by the thyroid. Using chicks, they find an immediate discharge of iodine already stored in the gland followed by a progressive increase in total iodine content upon injections of thyrotropin at 12 hour intervals. No increase was noted until the second injection when the iodine content was 100% above the control value. By 96 hours 500% more iodine was present in the glands. They also state that the collection of I ¹³¹ did not increase in proportion to the increased weight of the glands. In an effort to explain the increased iodine uptake, they went on to determine the rate at which labeled iodine was lost from the gland in response to thyrotropin treatment. Newly hatched chicks were given a single injection of the pituitary substance. By 24 hours 77% of the labeled iodine had left the thyroid, while at 72 hours, 96% had been lost. Since thyrotropin accelerates the rate at which iodine leaves the gland, the increased uptake could be explained on the basis of a greater "want" for iodine rather than a direct influence on its collection.

The effect of hypophysectomy on the iodine content of the thyroid has been studied by Taurog, Chaikoff and Bennet (1946). They report no change in the total thyroid iodine

content of rats lacking a pituitary and in fact, greater amounts of thyroxine were present. Two rats that were maintained for one year following removal of the pituitary showed no change in total thyroid iodine or thyroxine iodine as compared with controls. The gland size was greatly reduced, however. Three days after hypophysectomy, plasma protein-bound iodine values had decreased some 50%. Morton et al., (1942) have determined the uptake of I ¹³¹ in thyroids of hypophysectomized rats and report that less iodine was collected but more than would be expected from simple diffusion. Fractionation of the iodine present revealed that most of that collected was in the organic state, the greater percentage in the diiodotyrosine fraction. This would again indicate that collection and utilization of iodine was not dependent upon the action of thyrotropic hormone but probably the induced demand due to acceleration of thyroxine release. Schultz and Turner (1945) state that the lack of thyrotropic hormone stimulus in the chick results in an atrophic thyroid, increased colloid in the gland acini and increased total iodine content. Dempsey (1944) also finds that thyrotropic hormone serves to release colloid into the blood while suppression of the pituitary stimulus results in accumulation of colloid in the rat thyroid gland.

The manner in which a balance between the pituitary and thyroid secretions is attained is not fully understood. DeRobertis (1941) has proposed the presence of a proteolytic enzyme system within the follicular lumina capable of hydrolyz-

ing the colloidal thyroglobulin, resulting in the release of thyroxine. The thyroxine then would be free to diffuse into the circulation under the influence of the existing concentration gradient. He has extracted such an enzyme from the colloid of single follicles of the rat thyroid. According to this concept, greater activity in the production of the thyroid hormone, under the stimulus of thyrotropin, would also cause a greater production of the proteolytic enzyme and hence a more rapid secretion of thyroxine. Cortell and Rawson (1944) have shown a direct tie-up to exist between thyroxine and thyrotropin. They find that the presence of excessive circulating thyroxine interferes with the response of the thyroid to injected thyrotropic hormone. In the hypophysectomized animal, as well, administered thyroxine depresses the response of the thyroid gland to exogenous thyrotropin. The possibility of a neural influence on the secretion of thyrotropin has not been eliminated. Uotila (1939) finds that section of the pituitary stalk somewhat inhibits the normal seasonal variation found in the activity of the thyroid. On the other hand, the cervical sympathetics appear to be unnecessary for normal thyroid function (Uotila, 1939a). In view of the evidence that increased amounts of light (or the length of the day light period at a particular time of year) influences thyroid activity (Elmer, 1938), it is quite probable that the innervation of the pituitary is a regulatory factor in thyroid function.

It may be mentioned that changes in the histology of

the pituitary with various levels of thyroid activity have also been noted by several authors. For example, Gordon, Goldsmith and Charipper (1945) report that, as found after thyroidectomy, thiourea-treated rat pituitaries appear to have a decreased number of acidophils, vacuolation and increased numbers and size of the basophilic cells. Since the thyrotropic hormone is believed to have its origin in the basophilic cells (Severinghaus, 1937), this finding would seem to indicate hyperactivity of those cells and a possible depression of the secretion from the acidophils. This substantiates the observation of Sharpless and Hopson (1940) who further found that increased iodine and thyroid material increased the acidophilic count and decreased the number of basophils, but only the thyroid material produced the normal percentage of basophilic cells. The rats used in their work had been rendered goitrous on an iodine deficient soy bean diet.

The physiology of the thyroid gland is far from being a closed chapter in the fields of science. Along with numerous others, the scientists whose work has been briefly sketched above have contributed much to our present knowledge, and will continue to do so. It is hoped that the contribution to follow may aid, if only in a small way, in the never ending quest for a little insight into a few of Nature's infinite secrets.

EXPERIMENTAL PROCEDURE AND RESULTS

General Procedure

White Leghorn cockerels were chosen as experimental animals for this work. They were obtained, at the age of one day, from a Southern Michigan hatchery and maintained on the standard Arcady Starter and Grower ration* (containing .0023% potassium iodide). Food and tap water was constantly available until 12 - 15 hours prior to sacrificing the animals, when the feed trays were removed from the cages to eliminate the further ingestion of iodide. The room temperature was regulated to $80 \pm 2^{\circ}\text{F}$. and the cages were equipped with hovers, adjusted to a temperature of 87° to 90°F . one-half inch beneath them, for the first two weeks of the experimental period. Artificial lighting was provided from 8 A.M. to 5 P.M. daily.

Two experiments were carried out. In both cases the chicks were separated into groups of equal weight receiving daily subcutaneous injections of 1, 2, 3 and 4 $\mu\text{gm.}/100 \text{ Gm.}$ body wt. of crystalline d,l-thyroxine. The thyroxine was dissolved in 0.1 Ml. of distilled water made slightly basic (pH. 9.0) with sodium hydroxide. This material, used throughout the procedure, was isolated from iodinated casein and purified by E. P. Reineke. All animals were sacrificed at the age of 32 days, some having undergone treatment for 28 days and others for the last 14 days only. Tracer

*Manufactured by The Arcady Farms Milling Company, Chicago, Illinois.

amounts of radio-active iodine (I^{131})* were administered subcutaneously at precisely timed intervals previous to killing. After weighing each bird, the thyroid glands were extirpated, cleaned of extraneous tissue and weighed to the nearest tenth of a milligram on a Roller-Smith Precision balance. The individual glands were then placed on small copper discs and allowed to dry at room temperature before evaluating the specific activity of the collected I^{131} . The counts were made with a Geiger-Muller counter having a thin mica end window. Following this, the glands of each group were pooled and determinations were made of the total iodine content using the method of Kendall (1928).

Experiment One

The chicks were received late in January and sacrificed during the latter part of February, a period of the year when the intact thyroid of chicks has been shown to be highly active (Reineke and Turner, 1945). At the age of 4 days, those animals treated for a period of 28 days received their initial injection of the thyroxine. The others were retained until 14 days prior to the completion of the work, being treated only during that period. One-half of the animals of each group received a tracer dose of I^{131} 96 hours before killing, while the remaining half was given the same dose (approximately 0.1 uc.) 48 hours later. These time intervals were selected to be reasonably sure that

*Procured from the Oak Ridge Laboratories in a weak bisulfite solution.

optimal conversion of the I^{131} to the organically-bound form had taken place. Several investigators suggest that the maximum uptake of I^{131} by the thyroid gland occurs at about 24 hours (Skanse, 1948; Perlman, et al., 1941) in chicks and as early as 4 hours following a tracer dose in rats (Morton, et al., 1942). Since Taurog and Chaikoff (1947) report that 95% of the I^{131} present in the thyroid glands of rats is organically-bound within 15 minutes after the injection and Leblond and Gross (1949) find an even distribution of I^{131} throughout the colloid of thyroid tissue slices 24 hours post-injection, we have assumed that 48 hours allows ample time for nearly complete conversion of the I^{131} to the organic form. The latter authors have also demonstrated that there is no exchange of bound iodine with radio-active iodine when introduced to thyroid tissue in vitro.

Table I summarizes the data concerning the influence of exogenous thyroxine on the body weight and thyroid gland weight and the relationship existing between the two factors. It is quite evident that the thyroid weights are significantly lower in all thyroxine treated animals when compared to the control group. Further, there appears to be little change in gland weight per 100 Gm. body wt. at the different levels of exogenous thyroxine. The 1 ugm./100 Gm. amount reduced the thyroid weight by 38% whereas the 4 ugm./100 Gm. level resulted in a 51% reduction in weight in groups treated for 14 days. The longer treatment period

TABLE I

BODY WEIGHT - THYROID WEIGHT RELATIONSHIP AT THE VARIOUS
LEVELS OF EXOGENOUS d,l-THYROXINE
(EXPERIMENT I)

Group	Number of Chicks	Daily Thyroxine (μ gm./100 Gm. Wt.)	Treatment Period (Days)	Mean Body Weight (Gm.)	Mean* Thyroid Weight (mg.)	Thyroid Wt. per 100 Gm. Body Weight (μ g.)
1A	18	1	14	266.0	15.2	5.73
1B	17	2	14	261.9	14.8	5.65
1C	18	3	14	228.6	9.6	4.20
1D	21	4	14	258.9	11.6	4.47
1E	17	1	28	259.9	11.7	4.72
1F	18	2	28	267.0	11.1	4.16
1G	17	3	28	247.6	8.3	3.33
1H	20	4	28	265.4	8.7	3.28
Control	19	---	---	233.7	21.5	9.18

*Wet gland weight.

seems to produce a slightly greater effect in this regard. This would indicate that as little as 1 $\mu\text{gm}/100 \text{ Gm.}$ of thyroxine daily exerts almost maximal inhibition of factors controlling the thyroid weight. It has been demonstrated by several authors that pituitary thyrotropin, when administered in excessive amounts, serves to increase thyroid weight while the glands of hypophysectomized animals become atrophic. The present data, then, shows that 1 $\mu\text{gm}/100 \text{ Gm.}$ of chick weight is sufficient thyroxine to almost completely inhibit thyrotropin secretion or its stimulus to thyroid gland weight maintenance. Since greater reduction was seen at the higher levels of exogenous thyroxine, however, one must assume a differential inhibition of the pituitary factor and that it is directly proportional to the amount of administered thyroxine.

The mean body weights of all treated animals is about 10% greater than in the control birds. Little variation was seen among the chicks receiving thyroxine and the weight difference is not sufficient to rule out experimental error.

Following the determinations of I^{131} activity present in the thyroid glands of the various groups, the rates at which the collected I^{131} was leaving the glands was established during the representative period between 48 and 96 hours after injecting the isotope. (See Appendix). As shown in Table II, the specific activity present in the glands at the earlier time is related, inversely, to the level of administered thyroxine. Again, the I^{131} uptake was

TABLE II

THE TURNOVER OF RADIO-IODINE (I^{131}) BETWEEN 48 AND 96 HOURS
FOLLOWING A TRACER DOSE
(EXPERIMENT I)

Group	Number of Chicks	Daily Thyroxine (μ gm./100 Gm. Body Wt.)	Treat- ment Period (Days)	Biologic* Half- Life (Hours)	% I^{131} Lost/Day Body Wt.	Cts./Sec. /100 Gm. Wt. 48 Hours Post I^{131}	Cts./Sec.** Lost/Day /100 Gm. Body Wt.
1A	18	1	14	417.47	3.90	0.362	0.0144
1B	17	2	14	336.57	4.83	0.187	0.0095
1C	18	3	14	Neg.	Accum.	0.172	Slight Accum.
1D	21	4	14	Neg.	Accum.	0.087	Accum.
1E	17	1	28	558.87	2.93	0.424	0.0126
1F	18	2	28	47.71	29.43	0.221	0.0638
1G	17	3	28	80.02	18.77	0.200	0.0209
1H	20	4	28	1332.7	0.51	0.102	0.0005
Control	18	--	--	99.71	15.37	0.761	0.1140

*Time taken for half the collected I^{131} to leave gland.

**Relative I^{131} activity lost (percent I^{131} lost/day between
48 and 96 hours times I^{131} activity accumulated at 48 hours).

significantly reduced at the low level of thyroxine whereas larger doses influence this factor to a lesser degree than would be expected. Prolonging the treatment period from 14 days to 28 days had little effect on the relative uptake of I^{131} . It is interesting to note the percent of I^{131} turned over per day. The animals receiving 1 ugm. of thyroxine for 28 days appear to turn out the collected I^{131} at a much slower rate than those at the 2 and 3 ugm. levels. It is only at the 4 ugm. dose that the turnover is again reduced. The percent lost per day is, in fact, greater at 2 and 3 ugm. levels than shown by the control animals. Since the uptake is greater at the low dose level of thyroxine but the turnover is retarded, it would seem that the I^{131} is being accumulated with regard to the other treated groups. This does not appear in the groups treated for 14 days only. The loss of I^{131} is increased at the low thyroxine dose and accumulation occurs in the latter groups. The turnover of I^{131} is generally much slower in the groups treated 14 days. The variations appearing between the animals undergoing treatment for the two periods of time would suggest that the adjustment of the pituitary-thyroid balance in the presence of added circulating thyroxine is not complete after two weeks.

Briefly stated, the studies with radio-active iodine show that exogenous thyroxine administered for a two-week period inhibits the turnover of iodine in the thyroid to a greater extent than when the treatment is prolonged for

twice the time. The relative uptake, however, is consistent in all treated animals but decreases as the thyroxine dose increases. This may be interpreted as a slow rate of adjustment of the animal's own thyroid secretion mechanism to the presence of added circulating thyroid hormone. Secondly, 1 ugm. of thyroxine/100 Gm. wt. daily over a period of 28 days depresses the rate at which collected I^{131} is lost from the thyroid gland to a greater degree than does 2 or 3 ugm./100 Gm. body wt. and about equally as much as 4 ugm./100 Gm. body wt. This is not the case when the chicks are treated for only 14 days; the depression of I^{131} turnover is directly proportional to the thyroxine dose level. Thirdly, all treated animals show a depression of both uptake and turnover of I^{131} when compared with the control animals.

In view of the findings brought forth in the I^{131} studies and their contrast to popular concepts concerning the effect of exogenous thyroxine on the metabolism of iodine by the thyroid gland, it was of further interest to determine the total iodine content of glands from chicks treated for the two periods of time at differing dose levels. It must be pointed out that we did not attempt to distinguish between the inorganic and organically-bound iodine contained in the thyroid tissue, but have assumed the former value to be low and sufficiently consistent to validate this method of treatment. A number of investigators have established the inorganic iodine content of glands under various influences to be from 3 to 5% of the total iodine (Taurog and

Chaikoff, 1947; Morton, et al., 1942; Chaikoff and Taurog, 1949).

A summary of the total iodine data may be found in Table III. The dried thyroids of each group were subjected to digestion with potassium hydroxide, release of bound iodine and subsequent thiosulfate titration with starch as an indicator. The thyroid iodine is expressed as percent of the dry thyroid tissue by weight and micrograms of thyroid iodine per 100 Gm. body wt. In viewing the latter figures, it is found that the total iodine content parallels the uptake of I^{131} at all dose levels of thyroxine except the 4 ug./100 Gm. level. There appears to be a progressive decline from the highest value shown in group 1A (1 ug. of thyroxine/100 Gm. wt. for 14 days) to the lowest value in group 1C and again a higher amount of iodine in the group receiving the 4 ug. dose. The groups treated 28 days show a still higher iodine content of the low thyroxine level and a reduced amount at the next level. Unfortunately, the iodine analyses were not obtained for groups 1C and 1D. The control animals had more thyroid iodine per 100 Gm. body wt. than any of the treated groups. These determinations seem to be in accord with the indications that the turnover of I^{131} was reduced in the groups receiving the 1 ug. thyroxine dose, thus allowing the building of a larger total pool of iodine within the gland. With increasing amounts of thyroxine the turnover rates were faster, except in the case of the higher level, and this is borne out in the total iodine pool found in the glands.

TABLE III

TOTAL THYROID IODINE DETERMINATIONS
(EXPERIMENT I)

Group	Number of Chicks	Daily Thyroxine (ugm./100 Gm. Body Wt.)	Treatment Period (Days)	Total Thyroid Iodine (ugm.)	Iodine % Dry Thyroid Wt.	Thyroid Iodine /100 Gm. Body Wt. (ugm.)	Iodine Lost/Day /100 Gm. Body Wt. (ugm.)	Apparent** d,l-Thyroxine Lost/day/100 Gm. Body Wt. (ugm.)
1A	18	1	14	0.412	0.617	8.61	0.336	1.027
1B	17	2	14	0.295	0.504	6.63	0.320	0.978
1C	18	3	14	0.236	0.465	5.73	Accum.	Accum.
1D	21	4	14	0.383	0.639	7.04	Accum.	Accum.
1E	17	1	28	0.467	0.861	10.57	0.310	0.948
1F	18	2	28	0.235	0.504	4.89	1.439	4.400
1G	17	3	28	---	---	---	---	---
1H	20	4	28	---	---	---	---	---
Control	18	---	---	0.671	0.671	15.11	2.326	7.113

*Computed from percent I^{131} lost/day (Table II) times thyroid iodine/100 Gm. body weight.

**Computed from percent iodine per molecule of thyroxine. Figures must be halved for l-thyroxine secretion rate (See text).

According to the general concept, however, and if the gland weights can be used as a criterion of thyroid activity, both the turnover rate and iodine pool size should have fallen within narrower limits and would be expected to decrease as the amount of thyroxine given daily was increased. Since these figures are more complete in the experiment to follow, we shall delay a further consideration of these points until later. It will be noticed that the amount of iodine turned over per day has been calculated on the basis of the percent I^{131} turnover daily and the total iodine content of the thyroid on a body wt. scale. It was then possible to estimate the secretion rate of thyroxine, assuming any iodine leaving the gland was bound as thyroxine. This, again, will be discussed at a later time.

Experiment Two

The procedure followed was substantially the same as already indicated in experiment one. The animals were used during the month of March and kept under conditions outlined in the section concerned with general procedure.

A comparison of the mean body weights of this series of chicks (Table IV) with the former series (Table I) shows their failure to attain on equal gain although all birds were sacrificed at the age of 32 days. It was noticed, further, that the total feed consumption of this series was some 25% below that of the chicks included in the first experiment. In all other respects, however, the results appear to be

TABLE IV

BODY WEIGHT - THYROID WEIGHT RELATIONSHIP AT THE VARIOUS
LEVELS OF EXOGENOUS d,1-THYROXINE
(EXPERIMENT II)

Group	Number of Chicks	Daily Thyroxine (ugm./100 Gm. Wt.)	Treatment Period (Days)	Mean Body Weight (Gm.)	Mean* Thyroid Weight (Mg.)	Thyroid Wt. per 100 Gm. Body Weight (Mg.)
2A	22	1	14	199.4	8.2	4.12
2B	17	2	14	212.4	7.6	3.57
2C	21	3	14	212.4	7.8	3.67
2D	21	4	14	212.4	10.1	4.73
2E	21	1	28	198.6	10.3	5.67
2F	21	2	28	203.9	7.0	3.44
2G	17	3	28	190.9	7.7	4.03
2H	19	4	28	208.3	7.3	3.50
Control	24	---	---	212.6	17.2	8.18

*Wet gland weight.

reasonably comparable.

As seen in Table IV, the thyroid gland weights/100 Gm. body wt. are essentially equal regardless of thyroxine dose or period of treatment. Again it is pointed out that the 1 ugm. level of exogenous thyroxine seems to inhibit thyroid weight, presumably through inhibition of thyrotropin stimulation, to an almost optimal degree. A 40 to 50% reduction from the control value is evident at this low level of thyroxine administration and further reduction as the amount of administered thyroxine increases is negligible. No difference existed between the mean body weights of the treated animals or the control group.

The tracer dose of radio-active iodine was increased to about 0.7 uc. per chick as compared with the 0.1 uc. administered in the first experiment. This amount has been shown not to alter thyroid function in any way by Skanse (1948). The specific activity detected in the individual thyroid glands was thus increased with respect to the first series of birds.

As already shown, the relative specific activity of the I^{131} present in the glands 48 hours following the injection of the isotope was reduced as the amount of exogenous thyroxine increased (Table V). This was the case with both the 14 day period of treatment and the 28 day period and all values are below that indicated by the control group.

The same pattern of percent I^{131} turnover and relative specific activity lost per day was demonstrated by the groups

TABLE V

THE TURNOVER OF RADIO-IODINE (I^{131}) BETWEEN 48 AND 96 HOURS
FOLLOWING A TRACER DOSE
(EXPERIMENT II)

Group	Chicks	Daily Thyroxine (ugm./100 Gm. Body Wt.)	Treat- ment Period (Days)	Biologic* Half- Life (Hours)	% I^{131} Lost/Day /100 Gm. Body Wt.	Cts./Sec. /100 Gm. Wt. 48 Hours Post I^{131}	Cts./Sec.** Lost/Day /100 Gm. Body Wt.
2A	11	1	14	166.59	9.52	0.916	0.0092
2B	9	2	14	37.28	36.02	0.633	0.2268
2C	12	3	14	72.95	20.40	0.764	0.1520
2D	11	4	14	72.11	20.61	0.354	0.0735
2E	11	1	28	336.57	4.83	1.021	0.0493
2F	9	2	28	78.57	19.09	0.731	0.1482
2G	10	3	28	45.87	30.45	0.732	0.2190
2H	10	4	28	613.27	2.71	0.246	0.0075
Control	12	--	--	85.66	17.67	1.211	0.2178

*Time taken for half the collected I^{131} to leave gland.

**Relative I^{131} activity lost (percent I^{131} lost/day between 48 and 96 hours times I^{131} activity accumulated at 48 hours).

treated for the two time intervals. The turnover was greatly reduced at the 1 ugm. level of thyroxine, about equal to the control group at the 2 ugm. level, although the relative uptake was somewhat less, again reduced at the 3 ugm. level and substantially lower at the 4 ugm. dose level. It is interesting to again find that the percent turnover/day of I^{131} collected at 48 hours was much greater at the 2 and 3 ugm. levels of thyroxine (and with the 4 ugm. dose in group 2D) than the control animals show. The possible significance of this factor will be taken up at a later time.

The determination of the total iodine content of the thyroid glands (Table VI) indicates that the size of the total pool decreases as the level of exogenous thyroxine increases and appears to be proportional to the uptake of I^{131} with the exception of groups receiving 4 ugm./100 Gm. wt. Since the iodine pool is increased but the uptake of I^{131} is decreased when compared to the groups receiving lesser amounts of thyroxine, it is quite possible that a considerable portion of the total thyroid iodine is not labeled with radio-active iodine at this dose level. From reported thyroxine secretion rates of normal chicks, there is little doubt that 4 ugm. is above the daily output of the thyroid gland (Schultz and Turner, 1945; Reineke and Turner, 1945; Boone, Davidson and Reineke, 1950). We can then assume that the thyrotropin factor is completely inhibited due to this high level of circulating thyroxine. Taurog, Chaikoff and Bennet (1946) have shown that the total iodine content of the atrophic thyroid

TABLE VI
TOTAL THYROID IODINE DETERMINATIONS
(EXPERIMENT II)

Group	Number of Chicks	Daily Thyroxine (ugm./100 Gm. Body Wt.)	Treat-ment Period (Days)	Total Thyroid Iodine (Mg.)	Iodine % Dry Thyroid Wt.	Thyroid Iodine /100 Gm. Body Wt. (ugm.)	Iodine* Lost/Day /100 Gm. Body Wt. (ugm.)	Apparent** d, l-Thyroxine Lost/day/100 Gm. Body Wt. (ugm.)
2A	22	1	14	0.388	0.694	9.05	0.862	2.636
2B	17	2	14	0.209	0.509	5.79	2.085	6.376
2C	21	3	14	0.224	0.461	5.02	1.024	3.131
2D	21	4	14	0.410	0.667	9.18	1.893	5.233
2E	20	1	28	0.491	0.919	11.80	0.570	1.743
2F	21	2	28	0.155	0.445	3.62	0.691	2.113
2G	17	3	28	0.129	0.384	3.98	1.211	3.703
2H	19	4	28	0.336	0.590	8.49	0.230	0.703
Control	24	--	--	0.672	0.772	13.17	2.327	7.116

*Computed from percent I¹³¹ lost/day (Table V) times thyroid iodine/100 Gm. body weight.

**Computed from percent iodine per molecule of thyroxine.

Figures must be halved for l-thyroxine secretion rate (See text).

glands of hypophysectomized rats may increase as much as 90% and the iodine is stored as protein-bound material. Schultz and Turner (1945) suggest the same to be true in the case of the chick and the present data would indicate agreement. The uptake of radio-active iodine, therefore, does not indicate the total iodine content of the thyroid under these particular conditions.

The turnover of iodine as computed from the percent turnover of I^{131} times the total iodine content of the glands shows that the amount leaving the thyroid per day is somewhat greater in animals treated for only 14 days while a reduction occurs when the treatment is prolonged to 28 days. Furthermore, the loss of total iodine is suppressed to a greater degree with the 1 ugm. level of thyroxine than when the amount of exogenous thyroxine is increased to 2 and 3 ugm. The 4 ugm. level becomes meaningless in view of the fact that the entire pool of iodine is not represented by I^{131} , as already stated. In this regard, the loss of specific activity was reduced at this dose of thyroxine.

The apparent thyroxine secretion rate was calculated in terms of the loss of total iodine (See Appendix). Although consideration of this point will be reserved for the discussion, it may be pointed out that the control value of 7.116 is entirely unreasonable and must represent the loss of iodine from the thyroid gland other than that bound as thyroxine.

DISCUSSION

A résumé of the data collected during Experiment II is contained in Table VII. These data are not only representative of those concerning Experiment I, but also more complete and we shall confine our discussion to this information with reference to points of agreement or disagreement found elsewhere.

It has been well established that thyroid gland weight is an indication of the degree of thyrotropin stimulation that it is subjected to and that this stimulation is inhibited by thyroxine. This principal is demonstrated when the normal secretion of thyroxine from the thyroid is limited by such goitrogenic compounds as thiouracil and finds use in procedures designed to estimate thyroxine secretion rates of a number of species. Thyroxine secretion is blocked, no check of the thyrotropin level in the plasma is in effect and thyroid hypertrophy results. If exogenous thyroxine is administered, however, the hypertrophy subsides; when the gland weight is equal to that of the normal control animals, the amount of exogenous thyroxine should represent the animals' own secretion of the hormone per unit of time. With this in mind, it would appear that as little as 1 μ gm. of exogenous thyroxine per 100 Gm. body wt. is sufficient to reduce the thyroid weight per 100 Gm. of chick to some 40 to 50% that of the control group (Table VII). As the amount of thyroxine is increased the reduction of gland weight is relatively little

TABLE VII
(EXPERIMENT II)

Exogenous* Thyroxine (ugm.)	Thyroid Wt.* (Mg.)	Cts./Sec.* 48 Hr. Post I ¹³¹	Thyroid* Iodine (ugm.)	% I ¹³¹ * Lost/ Day	Relative* I ¹³¹ Lost/Day (Cts./Sec.)
14 Day Treatment Period					
1	4.12	0.916	9.05	9.52	.0092
2	3.57	0.633	5.79	36.02	.2268
3	3.67	0.764	5.02	20.40	.1520
4	4.73	0.354	9.18	20.61	.0735
28 Day Treatment Period					
1	5.67	1.021	11.80	4.83	.0493
2	3.44	0.781	3.62	19.09	.1482
3	4.03	0.732	3.98	30.45	.2190
4	3.50	0.246	8.49	2.71	.0075
Control	8.18	1.211	13.17	17.67	.2178

*Per 100 Gm. Body Weight.

**Approximate percentage of thyroxine-bound thyroid iodine.

TABLE VII (continued)
(EXPERIMENT II)

Exogenous* Thyroxine (ugm.)	Thyroid* Iodine Lost/Day (ugm.)	Apparent* Thyroxine Lost/Day (ugm.)	Times** 0.27	Plus Daily** Exogenous Thyroxine (ugm.)
14 Day Treatment Period				
1	0.862	2.636	0.714	1.714
2	2.085	6.376	1.722	3.722
3	1.024	3.131	0.845	3.845
4	1.893	5.233	1.413	5.413
28 Day Treatment Period				
1	0.570	1.743	0.471	1.471
2	0.691	2.113	0.571	2.571
3	1.211	3.703	0.999	3.999
4	0.230	0.703	0.190	4.190
Control	2.327	7.116	1.921	1.921

*Per 100 Gm. Body Weight.

**Approximate percentage of thyroxine-bound thyroid iodine.

although some change is apparent as the dose is increased. We can assume, then, that an almost optimal inhibition of thyrotropin occurs at the 1 ugm. thyroxine level. This point is substantiated in the first experiment as seen in Table I.

The uptake of iodine (I^{131}) at 48 hours following its administration indicates that it decreases directly as the thyroxine dose given. This is generally true in terms of the total iodine content of the glands except in the case of the 4 ugm. level of thyroxine. It is quite apparent that the total pool of iodine in the thyroid is not represented by I^{131} when 4 ugm. of thyroxine/100 Gm. wt. is injected daily. It has been shown by Taurog, Chaikoff and Bennet (1946) that the thyroid total iodine may increase as much as 90% in the atrophic glands of hypophysectomized rats. The same is reported to occur in the chick. (Schultz and Turner, 1945). The former authors also found that the increased iodine was organically-bound. It is reasonable to assume that the larger pool of thyroid iodine at the 4 ugm. level of thyroxine administration represents an accumulation of organically-bound iodine. Although some turnover of I^{131} is still taking place, as indicated by the loss of specific activity, it can not be assumed that the entire pool of thyroid iodine is turning over. The I^{131} present in the gland should be incorporated as the organic substance as long as 48 hours following its administration. In fact, Taurog and Chaikoff (1947) found 95% of the I^{131} present in the thyroid glands of rats

organically-bound as early as 15 minutes after injecting the isotope. Since 48 hours should allow ample time for the excretion of inorganic plasma I^{131} , it is reasonable that the majority of the specific activity found in the gland at this time is in the organic phase and that any loss of I^{131} represents organically-bound iodine.

In regard to the percent I^{131} lost/day and the relative loss of specific activity (% lost times relative I^{131} present in the thyroid 48 hours post-administration), it was found that the I^{131} turnover was greatly inhibited at the 1 ugm./100 Gm. wt. dose in animals treated for 14 days and those undergoing the 28 day treatment period. The turnover increased considerably at the 2 and 3 ugm. levels and again reduced at the 4 ugm. level of thyroxine. The group at the 4 ugm. dose for 14 days seems to show a greater percent turnover than the general trend among other groups, but the actual loss of specific activity was nevertheless reduced as expected. The turnover figures are in accord with the total iodine values as well. Since the turnover of I^{131} was very slow in groups receiving 1 ugm. of thyroxine, one would expect an accumulation of iodine in the thyroid greater than in following groups and this is precisely the case. A more rapid turnover of the radio-active material in groups at the 2 and 3 ugm. dose levels likewise is shown by a relatively small pool of total iodine. Accumulation is again taking place in the thyroid glands of chicks receiving the highest daily dose of the thyroid hormone.

Because the uptake of I^{131} and the amount of total iodine within the thyroids is proportionate in all groups of chicks, except those treated with the 4 ugm. dose of thyroxine, as discussed above, the percent turnover of I^{131} should represent the percent turnover of the total iodine. We find the ratio of these two sets of figures in general agreement for groups at the 1, 2 and 3 ugm. dose level for both treatment periods. Put the question of why more iodine is leaving the glands per day when the thyroxine dose is increased from 1 ugm. to 2 and 3 ugm./100 Gm. of body wt. remains to be answered.

To go back, the inhibition of thyrotropin should increase as the amount of circulating thyroxine is increased. If we assume that all organic iodine leaving the thyroid is thyroxine and that the function of thyrotropin is chiefly a factor controlling the release of thyroxine and the relative size of the gland, then the loss of iodine from the gland, presumably representing organically-bound iodine, is not in accord with the various levels of administered thyroxine. The loss of iodine is very low when the animals receive 1 ugm. of thyroxine while the loss is greatly increased when this dose is doubled and tripled. According to the weights of the glands computed per unit of body weight, 1 ugm./100 Gm. is almost sufficient to reduce the size to the optimal degree and this can be interpreted to mean almost complete inhibition of thyrotropin. If this interpretation is correct then we would expect the loss of iodine to be reduced almost to a

minimum at the 1 ugm. dose of thyroxine, which we find to be the case. The increased rate of iodine loss at the 2 and 3 ugm. dose levels of thyroxine could only mean that iodine is turning over that is not bound as thyroxine.

Let us consider the apparent thyroxine secretion rate as determined from the relative turnover of iodine from the thyroid gland. (This calculation is based on the relative molecular weights of iodine and thyroxine. Therefore, the iodine weight lost per day times the factor 1.529 represents weight of thyroxine lost per day). Assuming that all the iodine leaving the gland is in the form of thyroxine, the secretion rate of the control group becomes over 7 ugm./100 Gm. wt./day. In the light of secretion rates of chicks, which have been tabulated in Table VIII, this value is obviously too high. It would appear that these chicks were secreting amounts of thyroxine about threefold greater than the well established figures determined by the amount of thyroxine necessary to inhibit thyroid hypertrophy following thiouracil administration. How, then, are we to account for the great amounts of iodine leaving the thyroid glands of the control animals of the present study?

According to Chaikoff and Taurog (1949), about 27% of the total iodine present in the thyroid is bound as thyroxine while 70% is in the form of diiodotyrosine and about 3% remains in the inorganic state. It may be suggested that not only thyroxine iodine is secreted, but diiodotyrosine as well and, on this basis, 27% of the secreted iodine represents

TABLE VIII

REPORTED d,1-THYROXINE SECRETION RATES OF VARIOUS
STRAINS OF COCKERELS

Strain	Mean Body Weight (Gm.)	Thyroxine Secretion Rate (ugm./Day /100 Gm. Wt.)	Month Determined
White Leghorn ¹	246.0	2.39	Mar.
White Plymouth Rock ¹	277.0	1.98	Mar.
White Plymouth Rock ²	99.6	1.95	Feb. & Mar.
Slow Feathering ³ Rhode Island Red	140.0	1.55	Feb. & Mar.
Fast Feathering ³ Rhode Island Red	122.0	1.62	Feb. & Mar.

¹Schultz and Turner (1945).²Reineke and Turner (1945a).³Boone, Davidson and Reineke (1950).

thyroxine. The calculation yields a secretion rate of approximately 1.921 $\mu\text{gm.}/100 \text{ Gm. wt.}/\text{day}$ for the control group. The control group for Experiment I is exactly the same, as seen in Table III, and rather consistent with reported chick thyroid secretion rates. When applied to the thyroxine-treated groups (Table VII), the secretion rate plus the daily amount of exogenous thyroxine in groups receiving 1 $\mu\text{gm.}/100 \text{ Gm.}$ daily appears to approach the secretion rate of the control animals. It is recalled that the thyroid gland weights of these groups were reduced to an almost optimal degree and the thyroxine secretion rate would justify the assumption that the level of circulating thyroxine is nearly sufficient to completely inhibit the stimulation of the thyroid gland by thyrotropin, thus allowing partial atrophy and reduced weight of the gland. Further, the thyroid weight/100 Gm. body wt. of the group receiving the 1 $\mu\text{gm.}$ dose of thyroxine for 14 days is less than that of animals undergoing comparable treatment for 28 days. The computed secretion rate of the former group is greater and, therefore, represents a greater inhibition of thyrotropin stimulation of the gland which explains the relatively smaller gland size. It is also recalled that the gland weights of groups receiving the 2 $\mu\text{gm.}$ level of thyroxine were reduced with respect to those receiving half the thyroxine dose. Since the computed thyroxine secretion rate plus the administered material does not equal the computed secretion rate of the control group for animals at the 1 $\mu\text{gm.}$ dose level, it would appear that a daily amount of exogenous

thyroxine somewhere between 1 and 2 $\mu\text{gm.}/100 \text{ Gm. body wt.}$ are sufficient to completely block the thyrotropic factor.

We can then conclude that the blockade of thyrotropin by thyroxine is nearly complete in chicks treated with a supplement of 1 $\mu\text{gm.}/100 \text{ Gm. wt. daily.}$ This point is substantiated by a low turnover of iodine. When the exogenous thyroxine is increased to 2 and 3 $\mu\text{gm.}$, however, the rate at which iodine is leaving the thyroid gland is significantly increased and could only be explained in terms of a greater secretion of diiodotyrosine. When the level of circulating thyroxine reaches a higher value, this secretion is likewise inhibited and the accumulation of greater amounts of total thyroid iodine stands as evidence along with the decreased turnover of I^{131} . As already mentioned, the calculated secretion rate of the groups receiving 4 $\mu\text{gm.}$ of thyroxine is not reliable in view of the fact that all the thyroid iodine is not labeled with I^{131} .

In summary, it is suggested that organically-bound iodine is secreted from the thyroid gland both as thyroxine and diiodotyrosine and in proportions equal to the quantity of each compound found in the gland. The amount of iodine leaving the thyroids of normal control animals, as determined by the turnover of I^{131} and the total iodine content of the glands, is too great to account for thyroxine alone, but agrees with reported secretion rate values if only about 27% is considered to be bound as thyroxine. On this basis, the thyroid secretion plus the exogenous thyroxine of groups receiving

1 $\mu\text{gm.}/100 \text{ Gm. wt.}$ daily approaches the total secretion of normal chick thyroids. As evidence, suppression of gland weight to nearly optimal limits and the retarded turnover of I^{131} indicate a high degree of thyrotropin inhibition in these groups. As the dose of thyroxine is increased the turnover of iodine reaches a high figure. This is interpreted to mean that the majority of this iodine leaves the thyroid as diiodotyrosine under the influence of thyrotropin stimulation too limited to effect the secretion of thyroxine. If the exogenous thyroxine dose is increased to 4 $\mu\text{gm.}/100 \text{ Gm. wt.}$ per day then the secretion of all iodine from the gland is virtually eliminated; the total iodine pool indicates substantial accumulation and stagnation and the turnover of I^{131} is reduced to a minimum.

SUMMARY AND CONCLUSIONS

The effect of relatively small amounts of exogenous thyroxine on the iodine turnover of the chick thyroid gland has been studied. The relative uptake and turnover of radio-active iodine (I^{131}) and the total iodine content of thyroids from animals receiving daily, subcutaneous injections of 1 to 4 μ gm. of d,l-thyroxine per 100 Gm. body wt. was determined. Treatment periods of 14 and 28 days were employed.

The following conclusions were reached:

- 1) Nearly optimal suppression of thyroid weight was attained with as little as 1 μ gm. of thyroxine /100 Gm. wt. The phenomenon was attributed to inhibition of thyrotropin stimulation of the glands.
- 2) The relative uptake of I^{131} is reduced as the thyroxine dose is elevated.
- 3) The turnover of I^{131} is suppressed to a greater degree with the 1 μ gm. level of thyroxine than with 2 and 3 μ gm. amounts, but further reduction is evident at the 4 μ gm. dose level.
- 4) The total iodine content of the thyroids diminishes as the thyroxine dose increases, except that apparent accumulation of iodine occurs when the 4 μ gm. dose is employed.
- 5) The apparent thyroxine secretion rates, calculated from the % turnover of I^{131} daily and the total

thyroid iodine values, are about threefold greater in normal chicks than reported secretion rates determined by other methods. Assuming only part of the organically-bound iodine is secreted as thyroxine, the secretion rate becomes reasonable.

- 6) It is proposed that both thyroxine and diiodotyrosine are secreted from normal chick thyroids and in proportions about equal to the ratio of iodine bound as thyroxine (27%) and diiodotyrosine (70%) within the gland.
- 7) Qualification of this proposal is discussed with relation to gland weights, uptake and turnover of I^{131} and the total thyroid iodine of the thyroxine-treated animals.

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APPENDIX

A. Calculation of I^{131} Turnover

1. Turnover rate constant (K)

$$K = \log \frac{C_2}{C_1} \times \frac{2.3}{(t_2 - t_1)}$$

Where: C_2 = Counts/sec./100 Gm. wt. at t_2 (96 Hrs.)

C_1 = Counts/sec./100 Gm. wt. at t_1 (48 Hrs.)

2. Biologic half-life ($t_{1/2}$)

$$t_{1/2} = \frac{.693}{K}$$

3. Percent turnover per day

$$\% \text{ turnover/day} = 100 - (\text{antilog } \frac{2.4K}{2.3} \times 100)$$

B. Supplementary Data

1. Experiment one

<u>Group</u>	<u>Cts./Sec./100 Gm. Wt. at 96 Hrs.</u>	<u>K</u>
1A	.3342	.00166
1B	.1693	.00206
1C	.2096	Neg.
1D	.1587	Neg.
1E	.3997	.00124
1F	.1098	.01452
1G	.1319	.00866
1H	.0995	.00052
Control	.5447	.00695

2. Experiment two

<u>Group</u>	<u>Cts./Sec./100 Gm. Wt. at 96 Hrs.</u>	<u>K</u>
2A	.7500	.00416
2B	.2591	.01859
2C	.4842	.00950
2D	.2231	.00961
2E	.8952	.00206
2F	.5113	.00882
2G	.3541	.01512
2H	.2331	.00113
Control	.8213	.00809

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