STUDIES OF THYROID FUNCTION IN THE CHICKEN

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ABSTRACT

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by Ajit Singh

The thyroid secretion rate (TSR) in chickens of different ages was determined by four methods: (I) goiter prevention, (II) thyroid hormone substitution, (III) direct output and (IV) thyroxine degradation.

In method I, the daily dose of thyroxine, which gave thyroid weight of tapazole-treated birds equal to the untreated controls, was taken as the TSR. In method II, TSR was measured by determining for each group the dose of thyroxine (T_4) or triiodothyronine (T_3) required daily to maximally block I^{131} release in normal or tapazole-treated chickens. In method III, thyroidal I^{131} output rate was determined by daily counts, the thyroids were removed, analysed for total iodine and TSR was estimated as the product of daily I^{131} output rate (K_4) x thyroidal iodine x 1.529 (T_4 iodine equivalent). In method IV, half life ($t_{1/2}$) of T_4 was determined from successive plasma counts at 3-hour intervals after I^{131}-T_4 administration. Thyroxine distribution space (TDS) was calculated. Protein-bound iodine (PBI) was

analysed and extrathyroidal thyroxine (ETT) estimated as PBI x 1.529 x TDS. TSR was estimated as the product of ETT x K. Method IV was also applied to determine parameters of triiodothyronine degradation. TSR of bobwhite quail and coturnix was estimated by method IV.

The TSR values obtained in different experiments within each method were sufficiently close to indicate good repeatability. The representative TSR of chickens estimated by I, II, III and IV methods, respectively, were 2.28, 2.00, 1.10 and 2.03 μg/100 g/day. TSR of adult chicks, goitrogen-treated chicks, bobwhite quail and coturnix as estimated by method IV averaged 1.59, 1.02, 2.49 and 2.78 μg/100 g/day, respectively. TSR of goitrogen-treated chickens by method II was higher. There was no age difference in TSR measured by method III in growing chicks in the range of 1-9 weeks of age.

An increased plasma radioactivity was noticed in the 12-hour samples taken in the degradation experiments. This phenomenon seems to result from discharge of unchanged hormone from the liver.

Methods III and IV yielded lower TSR, TDS, ETT and thyroidal iodine content, but higher K_4, zero time percent uptake (U), and almost no difference in $t_{1/2}$ and PBI of iodine deficient chickens. Method II revealed no TSR

difference between chickens fed adequate or deficient iodine diets. Iodine deficiency retarded growth rate.

T_3 and T_4 were found to be equally potent in chickens by method II. The representative $t_{1/2}$ of T_4 in blood of chickens, bobwhite quail and coturnix were 3.23, 4.60 and 5.55 hours, respectively. The $t_{1/2}$ of T_4 was identical to that of T_3 in all birds. T_4 had a significantly greater $t_{1/2}$ in adult chickens.

The representative TDS of chickens, bobwhite quail and coturnix, respectively, were 29.39, 28.08 and 55.29 ml/100 g b.w. Adult chickens had lower TDS/unit b.w. T_3 distribution spaces of all birds were higher than of T_4.

The PBI and thyroidal iodine were analysed by using dilute ceric ammonium sulphate following alkaline ashing. The representative PBI of chickens, bobwhite quail and coturnix were 1.12, 1.76 and 1.26 µg % respectively.

Tapazole retarded growth rate, feather and comb growth of chickens. Two-three ug thyroxine /100 g/day counteracted these effects. Larger doses of T_4 depressed growth of normal and tapazole-treated birds. Thyroxine in small doses improved growth of normal chicks. Metabolic rate (M.R.) of chickens was determined in a closed circuit type manometric system. T_3 and T_4 produced a small and transitory rise in M.R.

The following indices of thyroid function were found to be significantly related: Age and K_4, age and thyroidal iodine, K_4 and thyroidal iodine, K'_4 and U, thyroidal iodine and body weight, T_3 distribution space and body weight.

These investigations support the conclusion that the thyroid function in chickens differs from that of mammals in lower PBI, shorter $t_{1/2}$ of thyroid hormones, their equal physiological potency and insignificant effect on M.R.

The author believes that the direct output and the T_4 degradation methods should measure true TSR in chickens provided all their known and unknown factors are properly accounted for. Consideration of the known factors strongly suggests that as presently applied, the direct output method most nearly represents the true TSR in chickens.

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INTRODUCTION

The thyroid gland is a major regulator of metabolic and productive processes. Study on chick thyroids is not only important from the standpoint of comparative physiology, but also to exploit economic traits of this species. Avian thyroid hormones are intimately related to the physiological processes associated with the energy metabolism, growth rate, feather and comb growth, molting, egg production, etc. etc. With recognition of meat and eggs as sources of high quality human nutrition and with increased development of poultry farming all over the world, it has become more pertinent to explore further in the fowl thyroid. This may help to step up greater and more efficient food production for the ever increasing human population.

It has been recognized that the thyroid status is best understood by quantitative measurement in terms of thyroid secretion rate (TSR). Other indices such as estimation of protein bound iodine (PBI), metabolic rate (M.R.), I^{131} uptake and release rates, and the triiodothyronine (T_3) test have been shown to give only qualitative information about thyroid gland function. Also, there are some marked differences of these indices between mammals and birds. Their

usefulness in measuring thyroid gland activity in chickens is thus even more questionable.

Previously only the goiter prevention and the T_4 substitution methods have been employed for measuring thyroid secretion rate in chickens. Their results indicated a large TSR difference. Furthermore, only meagre information is available about the other indices of thyroid function such as protein bound iodine (PBI), biological half life ($t_{\frac{1}{2}}$), thyroid hormone distribution space (TDS), thyroidal iodine uptake, release rate, thyroidal iodine content and the effects of the thyroid hormones on M.R., growth rate, feather and comb growth. Quail breeding has recently been gaining favour among the poultry farmers. However, practically no work has been reported on the thyroid function of this species.

In view of the above, it was proposed to study the thyroid function of chickens and quail by measuring their thyroid secretion rates. Investigations were also undertaken on related aspects, affording information on the thyroid hormone functions in these birds. In the present sutdy, the thyroid secretion rate of chickens was measured by four methods, viz. goiter prevention, the thyroid hormone substitution, the direct output and the thyroid hormone degradation (turnover) methods. The results of these methods were compared. Effects of dietary iodine content on various parameters of TSR and upon body and thyroid weights were

determined. Information was also sought on relative potencies of triiodothyronine (T_3) and thyroxine (T_4) in chicks. The effects of T_4 on growth rate, feather and comb growth, and that of T_3 and T_4 on M.R. were determined. Further $t_{\frac{1}{2}}$, TDS, extrathyroidal thyroxine and triiodothyronine (ETT), PBI and thyroidal iodine content were investigated in both chickens and quail. There are some, previously unknown, but important relationships between age, I^{131} daily output (K_4), and thyroidal iodine, between fractional output (K'_4) and zero time percent uptake (U), between thyroidal iodine, body weight and thyroid weight, and between body weight and TDS. These relationships have also been determined.

REVIEW OF LITERATURE

Variations within and between species, and the scientists' endeavor to ever improve upon methodology have led to the development of several methods for measuring thyroid secretion rate. This has brought about an enormous amount of literature, out of which, only the references pertinent to this project have been cited in the following review.

Goiter Prevention Method

The discovery of goitrogenic compounds enabled Dempsey and Astwood (1943) to develop a technique in rats for determining average thyroid secretion rate. Mixner, Reineke and Turner (1944) described a similar type of assay using one-day-old chicks. The method is based on the action of goitrogens inhibiting endogenous formation of the thyroid hormones in the test subject. This permits an increased output of TSH, which causes a compensatory enlargement of the thyroid gland. If, however, thyroxine is given exogenously, the TSH is held in check, whereby thyroid enlargement is prevented to an extent in proportion to the amount of thyroxine given. The daily dose of thyroxine needed to give thyroid weight of goitrogen-treated birds equal to the untreated

controls is taken as the adequate daily requirement of the hormone to maintain normal thyroid-pituitary balance and is considered as the secretion rate. The goiter prevention method has been most extensively employed for determining thyroid secretion rate in many species of small animals. Average levels of secretion equivalent to 2.70 and 0.75 μg dl-thyroxine daily were reported in two-week-old chicks. Maximum of this range was observed in fall and minimum in the summer season (Reineke and Turner, 1945).

Thyroxine Substitution Method

A large step forward in the development of methods for measuring thyroid secretion rate was made with the advent of radioactive iodine. Among the methods involving use of I^{131}, the thyroxine substitution method has been most widely used in all animals. This method is based upon the ability of the thyroid to rapidly accumulate a great proportion of ingested iodine, as also the fact, that administration of exogenous thyroxine will reduce both thyroidal I^{131} uptake and output. This suggested to Perry (1951), the possibility of an indirect thyroid assay by determining the amount of thyroxine required to block release of I^{131} from the thyroid. Perry used this procedure in rats and by varying dosage between different groups, he demonstrated a relationship between the amount of thyroxine given and the degree of inhibition of hormone release. Henneman, Griffin

and Reineke (1952) developed this method for measuring thyroid secretion rate in individual sheep. Furthermore, Reineke and Singh (1955) have devised a procedure based on thyroxine substitution whereby thyroid secretion rate could advantageously be measured in individual and smaller groups of animals than required earlier. The procedure involves the taking of external thyroid counts 2-3 days after I^{131} injections. Daily thyroxine dose is increased progressively at 2-day intervals, a thyroid count being taken before each increment in dosage. The end point then is the amount of exogenous l - thyroxine needed to block the I^{131} output from the thyroid. The latter measurement is made by expressing each thyroid count as percentage of previous count until the 100 percent point is reached.

In chickens, the thyroxine substitution method was first adapted by Pipes, Premachandra and Turner (1958). They reported thyroid secretion rate (TSR) values ranging from 2.0 to 5.0 µg l-thyroxine/ 100 gm/ day. Mellen and Wentworth (1959) adapted a modified procedure. They reasoned that the effect of a single injection of thyroxine in chickens may not persist for 24 hours and so they gave thyroxine in one, two or four equally spaced injections. From the average regression of thyroid radioactivity as percent of previous count on thyroxine dose, they estimated group secretion rates as 3.02 to 4.29 µg/ 100 g/ day. These results do not show any consistent difference in estimated

TSR due to injection schedule. In the same publication, Mellen and Wentworth (1959) reported results of another experiment where thyroxine was administered once daily and the dosage increased progressively at 48-hour intervals. The successive counts formed the basis of the individual regression of percent of previous count on thyroxine dose. By this procedure, they estimated TSR for individual chicks ranging from 3.25 to 5.17 µg/ 100 g/ day. Mellen and Wentworth (1960) again reported a comparison of chicks TSR by radioiodine and goiter prevention methods. The former method gave values from 3.5 to 4.5, while from the latter method, TSR was estimated as 1.40 µg/ 100 g/ day.

The workers using thyroxine substitution methods in chickens invariably mix a goitrogen in the feed to minimize reutilization of I^{131} from metabolized hormone and to accelerate release of the isotope from the thyroid. Reineke and Singh (1955) pointed out that when goitrogen is used in the substitution method, the estimated TSR is higher than that without goitrogen and that thyroxine fails to completely block I^{131} output from the thyroids of thiouracil-treated rats. On the other hand Turner et al. (1959) and Mellen (1961) believe that TSR is not affected by goitrogens. Himeno et al. (1961) compared thyroid secretion rate, measured with or without thiouracil in 4-week-old cockerels. They grouped the birds as that on (1) normal diet, (2) thiouracil mixed diet started 24 hours before substitution,

(3) thiouracil mixed diet started 48 hours before substitution, (4) thiouracil mixed diet started 120 hours before substitution. Average thyroid secretion rates in μg/100 g/day were obtained as (1) 4.61 (2) 7.00 (3) 7.97 (4) 11.67. These results are confusing, but nevertheless demonstrate that TSR in thiouracil-fed chickens increase significantly over those on normal diet. Also, the TSR increases further with length of thiouracil feeding. These authors believe that use of thiouracil is not adequate for precise determination of TSR in chickens. Reineke (1965) reported another influence of thiouracil on release rate. He noted that treatment with thyroxine prior to and during thiouracil administration blocked I^{131} output from rat thyroids for 3-4 days and then output was resumed at the same level as when thiouracil was given prior to thyroxine.

There has been little agreement among different workers on the end point reached, with regard to total or partial suppression of TSH in the substitution method. In the work with normal rats (Reineke and Singh, 1955) and sheep (Henneman, Griffin and Reineke, 1952) the daily thyroxine dose which maintained 100 percent previous count in the thyroid was taken as end point. In thiouracil-treated rats (Reineke and Singh, 1955) 92 percent previous count was taken as the end point. More recent data (Reineke and Lorschieder, unpublished) show that in rats on a 48-hour counting sequence thyroxine injections will only hold

thyroidal I^{131} at 97.5 percent of the preceeding count. Himeno and Turner (1961) took 95 percent previous count as their end point. They also recorded higher TSR in tapazole-treated birds than in normal birds.

Tanabe and Komiyama (1962) reported a modified thyroxine replacement method, based on partial inhibition of thiouracil-induced acceleration of I^{131} release from the chick thyroid. Daily TSR is measured as the amount of thyroxine which inhibits goitrogen induced acceleration of I^{131} release from the thyroid gland and returns the retention of thyroidal I^{131} to the rate before the start of thiouracil administration. A regression equation is solved for thyroxine dose vs. I^{131} retention rate. By this method, they estimated thyroid secretion in μg l-thyroxine/100 g/day as 1.50 to 1.80 for 6 to 7-week-old cockerels and 0.58 for 12-month-old hens. They noted that comparatively these values are 60 percent of those obtained by complete inhibition techniques; 85 percent of those obtained by the same procedure with non thiouracil-treated chickens, and close to the value derived from the goiter prevention assay.

Tanabe et al. (1965) compared effects of thiouracil, propylthiouracil and methimazole on thyroids and thyroxine metabolism in chicks. They reported that methimazole is the most potent and thiouracil the least with regard to I^{131} uptake and release. All these goitrogens at 0.1 percent level in the diet had little effect on thyroid secretion rate in

6 to 7-week-old cockerels. Further, thiouracil and propylthiouracil decreased (by 10 percent) deiodination of radiothyroxine and increased (by 20-30 percent) faecal excretion. On the other hand, neither methimazole nor $KClO_4$ had such effects.

Tanabe (1964) also observed a rough correlation between thyroid secretion rate and the levels of alkaline phosphatase in chicken serum. Wagai et al. (1965) determined thyroxine secretion rate in chicks by a microhistometric assay.

Direct Output Method

The direct output method for estimation of thyroid secretion rate in the rat was first worked out by Reineke (cited by Bhatnagar, 1963). This method involves measuring of thyroidal I^{131} turnover and the thyroidal iodine content. The product of these two parameters multiplied by a factor to account for the different activities of thyroxine and triiodothyronine gives an estimate of thyroid secretion rate in terms of T_4 released daily. No thyroxine or goitrogen is administered in this method.

Sorensen (1958) described a direct output method to determine thyroid secretion rate in cattle and pigs. Radioactivity of the thyroid gland was measured for a certain period after injection of I^{131}. In the same duration PBI and PBI^{131} were determined from the blood samples. The rate

constant for thyroidal I^{131} turnover is calculated from the declining radioactivity of the thyroid. The secretion rate of labelled hormone is found by multiplying the rate constant by the amount of thyroid I^{131}. Assuming that the specific radioactivity of the circulating thyroid hormones and that of the hormone just secreted is identical, thyroid secretion rate can be calculated as:

$$\frac{\text{Secreted hormone iodine } \mu g/hr.}{\text{Secreted hormone - } I^{131} \text{ percent dose/hr.}} = \frac{\text{Serum PBI } \mu g/100ml}{\text{Serum PBI}^{131} \text{ \%dose/100ml}}$$

Reineke (1964) compared the effect of iodine intake on thyroid secretion rate in rats as determined by thyroxine substitution and direct output methods. The results differed. In the substitution method, no significant difference was noted between groups given varying levels of iodine, while in the direct output method TSR values increased progressively with iodine intake.

No literature seems to be available involving computation of TSR in chickens by the direct output method.

Thyroid Gland Uptake and Release of I^{131}

Thyroid gland uptake and release of I^{131} have been measured to estimate thyroid functions. Their usefulness as indices of true thyroid secretion is uncertain. However, their values in qualitative and clinical thyroid conditions

are recognised. Goyings, Reineke and Schirmer (1962) suggested a method of diagnosis of hypothyroidism in dogs by measuring 24 hour thyroidal I^{131} uptake. They reported average ratios of thyroid to thigh counts as 11:1 and above in normal dogs and from 1:1 to 11:1 in the hypothyroid animals.

Recently Greenberg (1966) suggested the possibility of differentiating between primary and secondary hypothyroidism in humans by measuring the release rate of I^{131}. A rapid release suggests primary thyroid pathology whereas a slow release rate supports pituitary pathology as the cause of the thyroid insufficiency.

Turner et al. (1959) reported that the uptake of I^{131} by the thyroid is affected by many factors such as the size, weight and colloid content of the gland, variations in iodine content of the ration, kidney functions, state of pregnancy and lactation. Lodge, Lewis, Reineke and McGillard (1958) found no correlation between I^{131} uptake and estimated TSR in calves. Flamboe and Reineke (1959) did not observe a relationship either between TSR and percent uptake or I^{131} or between TSR and I^{131} output rate in the goat. However, in the sheep, Hoersch, Henderson, Reineke and Henneman (1961) observed a low negative correlation ($r = -.255$) between TSR and zero time percent uptake. They pointed out that the I^{131} uptake in itself is not a reliable quantitative estimate of thyroid hormone production.

Goitrogens decrease thyroidal retention of I^{131}, and if administered after the normal uptake, these drugs enhance release rate. Tanabe et al. (1965) reported a comparison of these effects of different goitrogens in chickens. March et al. (1964) observed that the thyroid gland and the thyroidal uptake of I^{131} were greater in chicks fed higher levels of protein. Supplementation of lysine in the diet, however, aggravated the amino acid imbalance and significantly depressed the thyroidal uptake of I^{131} per chick.

Thyroxine Degradation Method

The plasma thyroxine turnover or thyroxine degradation technique has been largely applied in man both in health and disease of the thyroid (Sterling et al. 1954, 1956; Ingbar and Freinkel, 1955; Gregerman, 1962). The technique comprises intravenous injection of a tracer amount of thyroxine. Serum or plasma radioactivity is measured in samples taken at different intervals. Biological half life, fractional turnover rate and thyroxine distribution space are calculated from the decline in radioactivity. Thyroxine degradation is then worked out from these parameters and the chemical PBI of the blood samples. The validity of this method depends upon the assumptions that for a steady state, hormone degradation is equivalent to hormone production. Furthermore, the administered I^{131} - tagged l - thyroxine behaves in vivo in precisely the same fashion as the natural

hormone secreted by the thyroid gland. However, in contrast with other methods, the turnover technique measures only thyroxine degradation and not the thyroxine equivalent of the biological effectiveness of secreted T_4 and T_3.

With regard to studies in man, Ingbar and Freinkel (1955) reported that in myxedematous patients, the volume of thyroxine distribution space (TDS) was significantly diminished and the fractional rate of thyroxine turnover was slightly reduced. Sterling and Chodes (1956) also made similar observations. They reported that extra thyroidal organic iodine pools, fractional turnover rate and the thyroxine degradation rate were diminished in myxedema and increased in thyrotoxicosis. Gregerman et al. (1962) employed the degradation technique in 73 euthyroid men of different ages. According to them, TDS decreased with age, but apparently only after decade 6. The fractional turnover rate also decreased with age but only till decade 7. The thyroxine degradation decreased by about 50 percent over the age span measured, roughly 20 to 80 years. The authors suggest that these age dependent variations may result from the decrease of metabolic rate with age. Recently Oddie, Meade and Fisher (1966) made a statistical analysis of human data published in 30 papers plus additional information communicated through numerous authors. They observed that sex and pubertal state showed no significant effect on thyroxine turnover. The thyroxine distribution space (TDS) increased

with increase in weight, but decreased in such clinical states as hepatitis and obesity. There is no effect of height or age on TDS. The fractional degradation is lowered as age advances. It is independent of weight and height. It decreases in hypothyroidism and hypometabolism (non thyroid) but increases in hyperthyroidism, hypermetabolism (non thyroid) and continuing antithyroid drug therapy. A significant increase in PBI occurs in hepatitis, in hyperthyroidism under treatment with antithyroid drugs, in hypothyroidism, nephrosis and diabetes.

The thyroxine degradation technique has been employed to estimate TSR in certain animals. Freinkel and Lewis (1957) first used it in sheep. Post and Mixner (1961) determined TSR in dairy cattle using two thyroxine turnover methods. They compared within animals, the results of (1) an isotope dilution procedure based on decline in specific activity of PBI following injection of I^{131} - labelled thyroxine, and (2) normal thyroxine turnover method based on the decline in plasma PBI after injection of non-radioactive thyroxine. The latter method was proposed earlier by Mixner and Lennon (1959). Daily thyroid secretion rates (per 100 lb body weight) estimated by (1) and (2) methods, respectively, averaged 0.40 and 0.39 mg in young calves and 0.14 and 0.13 mg in non lactating cows. These values compared favourably with those of the thyroxine replacement method. The authors stated that of the two turnover methods

the isotope dilution method is the most accurate. They also presented evidence that recycling of I^{131} through the thyroid during the first 96 hours does not influence significantly the turnover rate of I^{131} labelled thyroxine.

In rats, TSR obtained by the radiothyroxine turnover method (Gregerman, 1963) was generally in agreement with those gotten by other methods. The fractional turnover rate and thyroxine degradation were higher in female and in male and female rats exposed to cold. Thyroxine distribution space per unit body weight was found to be greater on exposure to cold and, unexpectedly, in old senescent rats. Since, changes in TDS reflect thyroxine degradation, the latter was also found to be increased in senescent rats. This finding is in contrast with the results obtained in man (Gregerman et al., 1962) and in rats reported by other workers employing other methods.

The degradation method has not previously been used for estimation of TSR in chickens.

Thyroid Function in Chickens

The function of the chick thyroid remains relatively unexplored. Although thyroid functions in birds and mammals are believed to be generally alike, nevertheless, the available literature points out important differences, which merit some discussion.

Both thyroxine (T_4) and triiodothyronine (T_3) have been isolated radiochromatographically from chicken plasma (Mellen and Wentworth, 1959a), and thyroid extracts (Shellabargar and Pitt-Rivers, 1958; Mellen and Wentworth, 1959a). Recently, however, Rosenberg et al. (1964) could not find T_3 in the hydrolysates of cockerel thyroids. They did not ascribe any reasons for this. Wentworth and Mellen (1961b) reported that the two thyroid hormones are found in blood of chickens, turkeys and ducks at the ratio of 60 percent thyroxine to 40 percent triiodothyronine.

The two thyroid hormones are reported to be bound to serum albumin. Unlike in mammals, the chicken and duck serums show no alpha-globulin-like thyroxine-binding protein (Ringer, 1965). Farer, Robbins, Blumberg and Rall (1962) found thyroxine-binding prealbumins and albumins, but no thyroxine-binding globulins in the blood of chickens, turkeys and pigeons. Tata and Shellabarger (1959) reported an equal binding affinity of albumin for T_3 and T_4 in chickens, but more recently, Heninger (1962) has shown that the two thyroid hormones are unequally bound to chicken plasma proteins. He observed that one hour following the injection of I^{131} - labelled hormones, 50.4 percent of T_4 and 24.8 percent of T_3 were bound to the albumin fraction of the plasma proteins. In vitro, T_3 was taken up by erythrocytes at a faster rate and to a greater extent than was T_4, thus showing that T_3 has a markedly lower affinity for plasma proteins than T_4.

A comparison of the protein binding of thyroid hormones in rat, chicken and human serum was drawn in the report of Dubowitz, Myant, and Osorio (1962). While noting a difference in binding of T_3 and T_4 by chicken serum, they also observed that chicken serum and rat serum resemble each other in their binding of T_3 and T_4 more closely than either of them resembles human serum, although relative potency of T_3 and T_4 in the rat is similar to that in man but differs in the chicken.

Owing to the protein binding differences, T_3 is expected to be more potent than T_4. However, the opinions differ. T_4 has been reported to possess less potency than T_3 in blocking TSH release (Gilliland and Strudwick, 1953). At variance with this, and to the situation in mammals, it was found that T_4 is equally potent as T_3 by the chick thyroid goiter prevention test (Shellabarger, 1955) and in stimulating heart rate (Newcomer, 1957). Furthermore, evidence suggests that T_4 is more potent than T_3 by such comparisons as (1) in reducing goiter (Newcomer, 1957; Mellen and Wentworth, 1959b), (2) radioiodine assay (Mellen and Wentworth, 1959b) and (3) in promoting oxygen uptake of chick myocardium (Newcomer and Barret, 1960). Protein binding is thus not an adequate explanation for differences in potencies of T_4 and T_3 in avian species (Heninger, 1962).

Metabolism and Excretion of Thyroid Hormones in Chickens

There is not much information available concerning metabolism and excretion of thyroid hormones in chicks. Recently, however, Hutchins and Newcomer (1966) reported that (1) the principal route of excretion of radioactive T_4 and T_3 was via bile instead of urine during a 4-hour collection period, (2) T_3 was excreted at a more rapid rate than T_4 via both bile and urine, (3) the principal metabolites of labelled T_4 and T_3 were conjugated and deiodinated thyronines present in the bile and I^{131}-iodide in the urine of chickens, (4) the percent of radioactive T_3 in chicken plasma decreased at a greater rate than did T_4, indicating that T_3 was metabolised peripherally and excreted at a greater rate than T_4.

Heninger and Newcomer (1964) reported that half lives of T_4 and T_3 in chicken plasma were almost identical, although in cardiac tissue, they noted that T_3 had a mean half life of 3.9 hours, while that of T_4 was 4.9 hours. In case of Japanese quail, McFarland, Yousaf and Wilson (1964) observed that the fractional turnover rate (k) of thyroxine was higher (4.02 percent loss/hour) in birds kept at $70^{\circ}F$ than that (2.56 percent loss/hour) in those kept at $90^{\circ}F$. Hypothalamic lesions in quail also decreased k values. In contrast to the short half lives of thyroid hormones in birds, it may be interesting to note that T_4 and T_3

respectively have half lives in man, 6.7 and 2.7 days (Sterling, 1955), in guinea pig 31.3 and 30.2 hours, (Ray and Premachandra, 1964); in rat for T_4 19 hours (Feldman, 1957) and in dairy cattle 46 to 48 hours (Post and Mixner, 1961).

Protein-Bound Iodine

Protein-bound iodine (PBI) is an estimation of the concentration of thyroid hormones in the blood. Using 4 week-old New Hampshire chicks, Bumgardner and Shaffner (1957) reported a mean value of 1.12 µg percent. They found no significant difference in PBI values between the controls and birds treated with thiouracil and thiouracil plus up to 8 µg T_4 per day. They also pointed out that repeatability of determinations of chick PBI was not good. Mellen and Hardy (1957) made comparisons between PBI levels of some birds and mammals. They reported values ranging from 1.13 to 1.22 µg percent in 8 and 20 months old chickens, and almost similar values in Pekin ducks. These values are far below those for rat, cow or man. Rosenberg et al. (1964) studied thyroidal metabolism of iodine (I^{127} and I^{131}) in chickens and rats by equilibration of injected iodine with existing thyroidal iodine. They reported an average PBI of 0.51 µg percent in 70-day-old cockerels maintained on low iodine diet. Supplementation of low iodine diet produced an

insignificant increase in PBI of chickens. (0.63 µg percent) In rats, however a high iodine diet produced a significant rise in PBI. Iodide supplementation also produced large increases in total and free iodide in both rats and chickens. Thyroidal content of iodide I^{127} in all animals was increased 3 to 4 fold by iodide supplemented diet. Rate of trapping of iodide was essentially the same in thyroids of rats on two kinds of diet. In thyroids of chickens on iodide supplemented diet, the rate was 5 - fold higher than in rats.

Effect on Growth and Metabolic Rate

That thyroidectomized chicks grow less than controls is well known, but levels of replacement therapy, which can bring back normal or near normal growth is not too clear. Winchester and Davis (1952) claimed to have stimulated growth of thyroidectomized chicks to 91 - 99 percent of control body weight by daily injections of 2 or 4 µg of dl-thyroxine/100 g.b.w. Almost similar results were obtained by Clegg, Ericson and Hein (1959).

Ringer (1965) made a comprehensive review on effects of thyroidectomy and hypothyroidism on growth of chickens. He stated that goitrogens have been used to increase growth or improve carcass quality through increased deposition of fat. The rationale of using goitrogens is to depress the

thyroid activity. This is then reflected in a reduced metabolic rate, which in turn could produce a gain in weight. Results contrary to the above have been obtained by some workers.

Chickens fed a ration with thiouracil at 0.2 percent level (Herbert and Brunson, 1957) and methimazole at levels above .001 percent (Wilson and MacLaury, 1961) showed a decrease in weight gains. Combining diethylstilbestrol with thiouracil, however, improved growth in chickens (Andrews and Bohren, 1947). Such combinations of stilbestrol with methimazole improved both growth and carcass quality in turkey broilers (miner et al., 1959).

Effect of thyroprotein on the growth of normal fowl is also not clear. White Plymouth Rocks fed less than 0.1 percent thyroprotein showed enhanced growth rate up to 6 weeks but not at 12 weeks (Irwin, Reineke and Turner, 1943). At other occasions, feeding of thyroprotein did not produce any growth gain (Boone, Davidson and Reineke, 1950), or the growth rate was even depressed (Turner, Irwin and Reineke, 1944; Oloufa, 1955). Recently, Snedecor and Camyre (1966) have shown an interaction of androgen and thyroid involving comb growth, but could not find any such clear effect on body weight of cockerels. Increased liver glycogen and liver weight following hypothyroidism was noted by Snedecor et al. (1964, 1965, 1966).

A single injection of thyroxine in mammals stimulates metabolic rate (M.R.) and the action is prolonged over a period of several days. Unlike this, Mellen (1958) reported that thyroprotein-fed birds show increased metabolic rate only for a short time during the first few hours after fasting. After 12-14 hours of fasting, M.R. was consistently low except at 22-24 hours. A depression in M.R. following thyroidectomy occurs in many avian species (Lee and Lee, 1937; Winchester, 1939; Marwin and Smith, 1943; Mellen and Wentworth, 1962). The stimulating effect of thyroprotein on M.R. in chickens lasts for as long as supplementation is maintained (McCartney and Shaffner, 1950). Increase in metabolic response proportional to graded doses of thyroprotein (at levels greater than 5 gm/cwt) was noted by Singh and Shaffner, (1950). They also reported that increasing the caloric value of the ration increased metabolic response to thyroprotein. Strite and Yacowitz (1956) while working out a modified method for measuring O_2 consumption of young chicks noted a slight rise in M.R. with thyroprotein.

MATERIALS AND METHODS

Birds and their Feed

A total of 399 white leghorn chicks and 46 quail were used in this study. The chicks were obtained as day-old cockerels from a single hatchery. They were placed in brooders adjusted to 35°C and kept on 14 hours per day lighting period. All the birds had free access to fresh drinking water. According to design of experiments, the chicks were fed ad libitum with the following rations:

1. Michigan State University 63-S chick starter krumbles. This is manufactured by King Milling Company, Lowell, Michigan. The formula is given in Appendix G. This was adequate with iodine.

2. Rat ration mixture SR-2, with iodine supplemented to provide 1.3 µg/gm of diet.

3. Rat feed mixture SR-2. This was deficient in iodine. The formula of this diet is given in Appendix H.

Bobwhite (Colinus virginianus) and Japanese quail (Coturnix coturnix japanica) were procured as adult male birds, 56 to 68-week and 10-week old, respectively, from the Poultry Science Department, Michigan State University. Twelve white leghorn roosters 56-week old, used in degradation

studies, were also obtained from the Poultry Science Department. The diets of those birds were standard quail and poultry rations containing adequate iodine (Appendices J and I).

Chemicals and Drugs

Methimazole (1-Methyl-2-Mercaptoimidazole, or tapazole) was obtained from Eli Lilly and Company. It was used as a goitrogen at the levels of 0.05 percent in the ration or 0.025 percent in drinking water.

Thyroxine and triiodothyronine, stock solutions of 100µg/ml of Sodium-L-thyroxine (merck) and triiodo-L-thyronine (Smith, Kline and French) were prepared and stored in the refrigerator for a short period of time. For equimolar quantities of the two hormones, 1.0 µg of T_4 is needed for each 0.84 µg of T_3. In preparing solutions, the crystalline hormones were first dissolved in a small amount of 0.1 N NaOH. Enough 0.1 N HCl was then added to make the solution slightly cloudy which signifies the point where the monosodium salt is formed. This suspension was diluted with an appropriate amount of normal saline solution to make up the desired concentration. The solutions for injection were made up as needed from the stock solution. In one experiment on metabolism, T_4 and T_3 solutions were mixed in the ratio of 60:40 for treating a group of chicks.

In tracer experiments, ten microcuries of carrier-free I^{131} as NaI, made up to a volume of 0.25 cc in normal saline solution, was injected subcutaneously to each bird. A drop of chicken plasma was added to the diluted I^{131} solution to minimize adsorption to glass. Standards containing 1/10 of the injected dose were kept in small glass planchettes. These were stabilized by addition of a drop of casein suspension containing an excess of Kl and $NaHSO_3$. The radioactivity contained in the standards was measured every time a determination was made on the thyroid and at similar geometry. Percent of the injected dose was then determined by comparison with the standards.

Radioactive thyroxine and triiodothyronine (triomet) labelled with I^{131} were obtained from the Abbot laboratories as 50 percent propylene glycol solutions. They had specific activities of 38.4 and 28.2 mc per mg, respectively. They were diluted with normal saline solutions containing a drop of chicken plasma and were administered intravenously in doses of 10 μc per chick and bobwhite quail and 5 μc per Japanese quail. The standards containing 1/100 to 1/50 of the dose in the same volume of fluid were kept in plastic vials similar to those used for the plasma samples. The standards were counted at the same geometry each time plasma samples were counted. After making correction for the background count, percent of the injected dose in plasma was calculated.

Standards were based on the amount of protein-precipitable radioactive material in the labelled hormones. Gregerman, et al. (1962) reported that thyroxine content of commercial solutions was approximately 90 percent. In this study also, it was observed that the recovery of counts after TCA precipitation of the plasma containing labelled hormone in 8 trials was 89.8, 90.9, 93.4, 92.4, 93.9, 91.0, 93.3 and 91.3 percent of the initial radioactivity. It was thus thought that a correction of the standards by an average factor of 0.919 is necessary, as otherwise, the distribution space measurements would be over estimated, since the impure component would probably be eliminated much more rapidly than the hormone.

Counting Apparatus and Procedure

The gamma radiation from the thyroid region of birds was counted on a scintillation counter (Nuclear Measurements Corporation) with a 2" crystal connected to a radiation analyzer and a laboratory scaler. A count rate meter was connected in the circuit to help in determining the position of the chick for the highest counts. The unanesthetized bird was placed in a specially improvised plastic cone. This cone served both as an immobilization apparatus and a device for location of the thyroid geometry for the maximum counts. Body background counts were taken over the thigh

region. Thyroid counts and the counts in the standards were corrected, respectively, for body and room backgrounds. Plasma samples and the thyroids when removed from the body were counted in a well type scintillation counter connected to a radiation analyzer and laboratory scaler.

Goiter Prevention Method

The goiter prevention assay described by Mixner, Reineke and Turner (1944) was employed on week-old cockerels. In one experiment 47 chicks were divided into 6 groups. One group served as a normal control while all birds in the other five groups were given 0.025 percent tapazole in drinking water. Out of these five, a group receiving no thyroxine served as a hypothyroid control. The remaining four groups received, respectively, 0.5, 1.0, 2.0 and 3.0 μg L-thyroxine per 100 gm b.w. daily for the duration of the experiment. On the 20th day, the birds were killed with ether and the thyroids dissected out cleanly and weighed on a Roller-Smith torsion balance. Thyroid weights in mg per 100 gm b.w. were plotted against thyroxine dosage (Figure 1). The thyroid secretion rate was determined by the dose of thyroxine at which the thyroid weight curve of the injected birds intercepted the normal thyroid weight line.

In this study, the original goiter prevention technique was modified such that all birds were weighed at 3-day

intervals to determine the effects of various treatments on growth rate. The results of the first experiment prompted the author to undertake a combined study on body growth, feather and comb development with the goiter prevention assay in the second experiment.

One hundred 1-week old chicks were divided into 10 groups. Group 1 served as the normal control. Groups 2, 3, 4, and 5 received, respectively, 0.5, 2.0, 3.0 and 4.0 μg L-thyroxine per 100 gm b.w. daily. Group 6 was put on tapazole water and served as hypothyroid control. Groups 7, 8, 9 and 10 received both tapazole water and daily injections of 1.0, 2.0, 3.0 and 4.0 μg L-T_4/100 gm b.w., respectively. The weights of all birds were regularly recorded. Growth of feathers was estimated by dying wing and tail feathers with picramic acid at the beginning and measuring the newly grown parts on the 12th, 20th and 32nd day. The comb height was measured only on the 32nd day when chicks were killed for TSR determination from their thyroid weights.

Thyroid Hormone Substitution Method

In general the T_4 substitution method of Reineke and Singh (1955) for determination of thyroid secretion rate in rats was adapted to chicks. Sixty male chicks aged 2 - 6 weeks were used in 6 experiments. The experiments were designed to test results of the thyroid hormone substitution method as follows:

1. Comparison with other methods of TSR determination.

2. Influence of dietary iodine intake in estimation of TSR.

3. Influence of goitrogens in estimation of TSR.

4. Determination of the comparative potency of T_3 and T_4 on normal and tapazole-treated chicks.

Forty-eight to seventy-two hours were allowed for maximum thyroidal uptake of I^{131} after administration of the isotope. The initial counts were taken and the injections of T_4 or T_3 were then started. Where effects of the goitrogens on the apparent TSR were to be determined, the birds were put on tapazole in feed or water on the day when hormone injections were started. The injections were given daily but counts were made on alternate days and after each count, the dose was increased gradually till the maximum percent previous counts were obtained. This dosage level of the thyroid hormones per 100 gm b.w. was taken as the daily TSR. Goitrogens enhance release rate of I^{131} from the thyroid and thus to avoid risk of losing considerable radioactivity from the thyroid, the increment in hormonol dose in certain cases was given daily after taking thyroid counts. This modification apparently had no effect in the chicken.

Direct Output Method

The technique of Reineke (1963, 1964) for rats was applied to birds. Sixty 1 to 9-week old cockerels were used

in 6 different experiments, with the idea of comparing:

1. results of TSR computation with that by other methods.

2. effect of age and iodine content in feed intake, thyroidal iodine content, thyroidal iodine turnover (release rate).

3. correlation, if any, between these different parameters.

Forty-eight to seventy-two hours after administration of I^{131}, radioactivity of the thyroid was determined daily consecutively for 5 to 6 days. Percent of the injected dose in the thyroid was plotted on the ordinate of semi-logarithmic paper against time in days on the abscissa. The output curve was fitted by inspection and extrapolated to time zero. The maximum uptake and biological radio iodine half-life were read from the curve. The birds were killed for recovery of thyroids, which were subjected to total iodine analysis. The following calculations are made for estimation of TSR.

I^{131} output rate constant: $k = \frac{.693}{t_{\frac{1}{2}}}$ or

$$k = 2.302 \frac{(\log At - \log Ao)}{t}$$

Fractional output daily (fractional turnover):

$$= k'_4 = 1 - e^{-kt} \quad (t = 1 \text{ day})$$

I^{131} daily output corrected for recirculating iodine: $k_4 = \frac{k'_4}{1 - U}$ U = Percent maximum uptake

$$= \frac{\text{Maximum uptake at zero time}}{100}$$

Daily iodine output = k_4 x total thyroidal iodine (From iodine analysis of thyroid)

Daily thyroxine output = Daily iodine output x 1.529 (thyroxine equivalent of iodine)

Daily TSR per 100 gm b.w. = Daily thyroxine output x $\frac{100}{\text{b.w. (gm)}}$

Rosenberg et al. (1964) did not find T_3 in the hydrolysates of chick thyroid. Mellen and Wentworth (1959a) observed 86 parts of T_3 and 14 parts of T_4 in thyroid digests at 96 hours after I^{131} injection. Shellabarger and Pitt-Rivers (1958) also reported that both T_3 and T_4 are present in the thyroids of chicks 24 hours after I^{131} injection, but that T_3 radioactivity does not exceed 5 percent of T_4 activity. In view of such disagreements, as also of the almost identical potencies of the two hormones as observed in our work on chickens and that reported by other workers, it was decided that the factor 1.53 which is applied for adjustment of T_4 equivalent in proportion to the potency of the mixture of T_4 and T_3, assumed to be released in rats, may not be applied in case of chickens.

Thyroid Hormone Degradation Method

In general the methods used in man (Ingbar and Frienkel, 1955; Sterling and Chados, 1954; Gregerman et al., 1962) were applied in birds. Fifty-four, six, seven and fifty-six-week-old chicks, twenty-six bobwhite and twenty Japanese quail, all males, were used in thyroxine and triiodothyronine degradation experiments. Six-week-old chicks were fed on diet deficient in iodine while all other birds were on diet adequate in iodine. The blood samples were taken from the wing vein opposite to the one used for administration of labelled hormones. Sufficient blood was drawn every 3rd, 6th, 9th and 12th hour to yield 0.5 cc and 0.1 cc plasma in case of chicks and quail, respectively. In some preliminary experiments on chickens, blood samples were collected 24 hours after administration of the tagged hormones, but no appreciable counts could be detected at that time. Thus sample collection at 3-hour intervals was resorted to. The last or 12-hour blood samples in many cases were collected by heart puncture. These samples were large enough for use both for counting radioactivity and for PBI determination. The percent of the injected dose in the plasma as calculated from each sample was plotted against time on semi-logarithmic graph paper (Figure 8). The curve was fitted by inspection and extrapolated to time zero. The maximum radioactivity at zero time, thyroxine distribution

space (TDS), biological half life ($t_{\frac{1}{2}}$), fractional turnover (k) were calculated.

In certain birds, especially the quail it was noticed that the samples obtained by heart puncture contained more radioactivity than the earlier samples obtained from the wing vein. A similar type of overdischarge was particularly well marked when the last samples were taken by decapitation of quail in certain earlier experiments not included for collection of this data. In view of this, the last samples in T_3 degradation experiments with quail, were taken both by venous and heart punctures and the percent radioactivity compared in the two. From this, percent discharge was calculated. In case of the subjects where fourth samples were collected only by heart puncture, percent increase in radioactivity was calculated from the difference between this point and that of the curve extrapolated from the earlier samples. The thyroxine turnover was calculated as below.

Thyroxine distribution space:

(in mls) $$(TDS) = \frac{100}{\% \text{ dose at 0 time in 1 ml. plasma}}$$

$T_4 - I^{131}$ turnover rate constant: $k = \frac{.693}{t_{\frac{1}{2}}}$ (hours)

Fractional turnover: $k = 1 - e^{-x}$

Daily thyroxine degradation or TSR = ETT x k x 24

(Multiply by 1.529 to convert thyroxine iodine into its equivalent as thyroxine.

Multiply by 24 to convert hourly degradation to degradation per day.)

Daily TSR in μg per 100 gm b.w. = Daily TSR x $\frac{100}{\text{b.w. (gm)}}$

Uptake of Thyroid Hormones by Red Cells

In order to study uptake of T_4 or T_3 by avian erythrocytes, a drop each of I^{131} labelled T_4 and T_3 was added into two equal chicken blood samples. Whole blood was counted and then incubated at the chicken body temperature (41.6°C) for 2 hours. Plasma was separated from the red cells after centrifuging. The cells were washed thrice with normal saline solution, centrifuged and the wash mixed everytime with the plasma. The cells and the diluted plasma were then counted and necessary corrections made for geometry and decay. Percent of the hormones bound to R.B.C.'s was determined.

Determination of Metabolic Rate (M.R.)

Reineke's multiunit metabolic apparatus, a modification of the Maclagan and Sheahan procedure was used for determining metabolic rate in chickens. This multiunit system consists of 12 desiccators connected by three-way stop cocks to mercury manometer, vacuum and oxygen lines. The animal is weighed and placed in a tightly sealed desiccator of known volume. The desiccator is charged with oxygen and contains soda lime for the absorption of CO_2. As O_2 is used, the pressure in the chamber decreases proportionately. O_2 consumption is computed directly from the decline in pressure

and the net volume of the chamber minus volume occupied by the animals.

Forty-eight 3 to 5-week old chicks were used in this study. In the first series of experiments, 24 birds were equally divided into 4 groups comprising controls, T_4, T_3 and a combination of T_4 + T_3-treated birds. Each treated bird received 6.0 µg/100 gm b.w. daily of either single or combined hormones. The birds were fasted for 12 hours before determination of M.R. Of the 4 determinations made in this series, two were done after 24 hours and one each 2 and 3 hours after the last hormone administration. The first determination was made 7 days after initiation of injection.

In the second series, two experimental groups of 8 birds each were injected subcutaneously with 4.0 µg/100 gm b.w. of T_4 and T_3. The third group containing the same number of birds was injected similarly with normal saline solution and served as a control. M.R. was determined 1, 2 and 7 hours after single injections of the hormones.

Calculations:

$$O_2 \text{ consumption (mls./Hour)} = (V - Va) \times \frac{P}{760} \times \frac{273}{273+t} \times \frac{60}{T}$$

Where V = net vol. of unit in ml.

Va= Volume of animal

P = Pressure difference in mm. Hg.

t = temperature in desiccator

T = Time of determination in minutes

Statistical Analyses

The following methods were used for statistical analyses of the data:

One- and two-way analysis of variance and linear regression for finding correlation coefficients and estimation of confidence intervals, means and standard errors were done by the methods given in Li (1964). The Mann-Whitney U test for comparison of two independent samples and the Spearman rank correlation coefficient for determining relationships between T_3 distribution space (TDS) and body weight of chicks, were done by the methods given in Siegal (1956).

RESULTS AND DISCUSSION

Goiter Prevention Method

TSR values (2.32 and 2.25 μg T_4/100g/day) from the two experiments on the goiter prevention method (Figure 1) are in close agreement. The slight difference is probably due to experimental variation. In the climate of Michigan, it is expected that there would be no seasonal variation during the span of time covered by the two experiments. Also, there were only 12 days age difference between the groups of birds used in the two experiments.

It may be noted in Figure 1 that the thyroid weights were greater in the group receiving tapazole plus daily injections of 0.5 μg T_4 than those in the group receiving tapazole only (39.40 and 38.95 mg per 100 g.b.w. respectively). This phenomenon is explained by the fact that in a critical dose range, T_4 potentiates goitrogenesis by a goitrogenic drug. Sellers and Schonbaum (1965) believed that this action of T_4 is mediated via an effect on the adenohypophysis or higher centres.

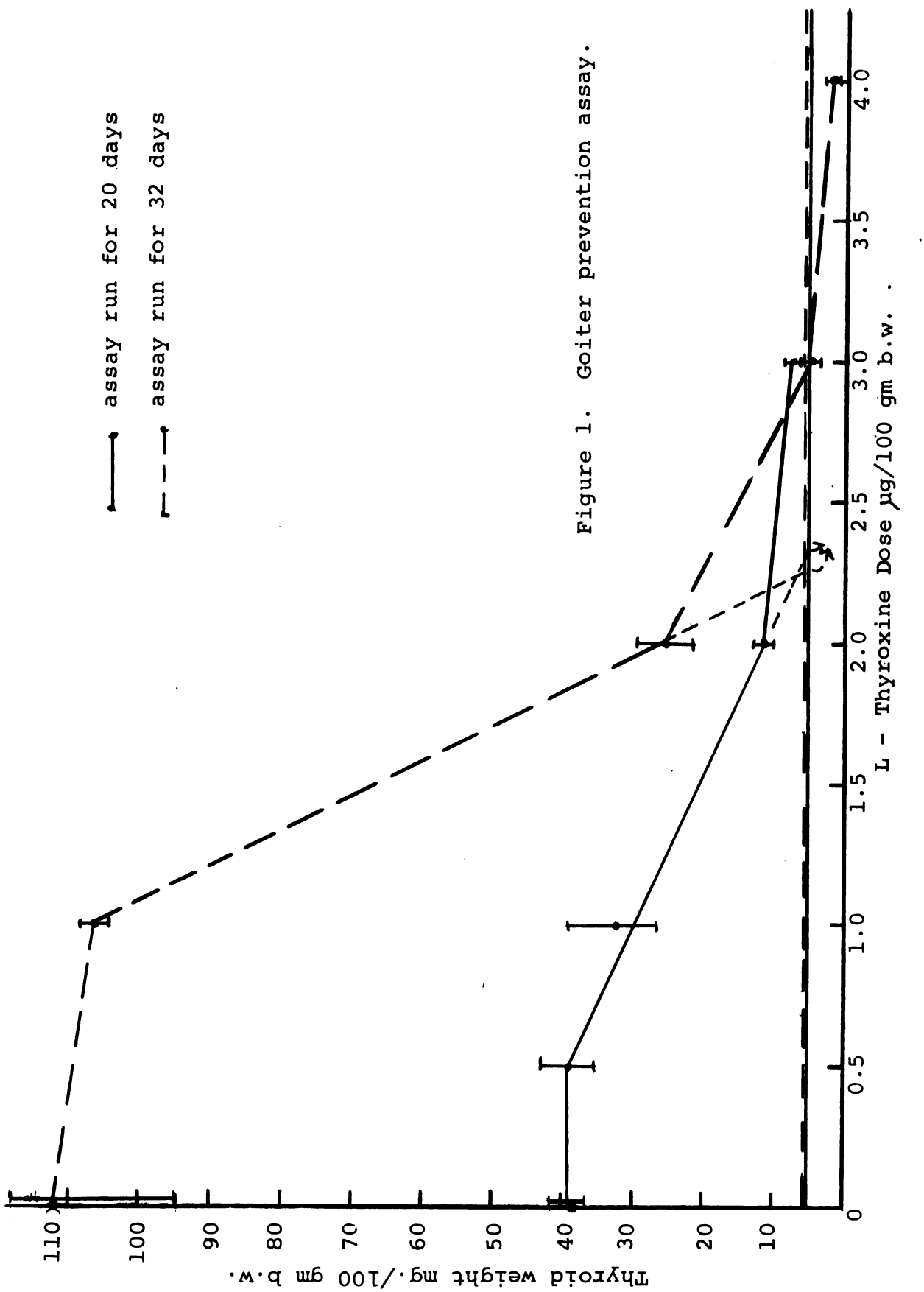


Figure 1. Goiter prevention assay.

Thyroid Hormone Substitution Method

Table 1 and Figure 2 show that the values of TSR obtained by substitution of T_4 and T_3 in normal birds (2.00 and 2.17 respectively) are significantly lower than those in the tapazole-treated birds (4.14 and 3.86 respectively).

Differences of TSR by the T_4 substitution method between normal and goitrogen-treated subjects were also observed in rats (Reineke and Singh, 1955) and in chickens (Himeno, Tanabe and Komiyama, 1961; Himeno and Turner, 1961). Turner et al. (1959) in rats and Mellen (1961) in chickens could not find TSR differences between normal and goitrogen-treated animals.

Table 1 also shows that for both T_4 and T_3, the end points of percent previous counts in the normal birds averaged higher than that in the goitrogen-treated birds. This again is supported by the work of Reineke and Singh (1955) and Reineke and Lorscheider (unpublished) in rats. Tanabe and Komiyama (1962) suggested a different end point in a modified substitution method in chickens. TSR in that procedure was estimated as that amount of T_4 which inhibits thiouracil-induced acceleration of I^{131} release from the thyroid gland and returns the retention of thyroidal I^{131} to the rate before start of the goitrogen. Ringer (1965) is of the view that the physiological basis for computation of TSR in the method of Tanabe et al. is not clear, since such

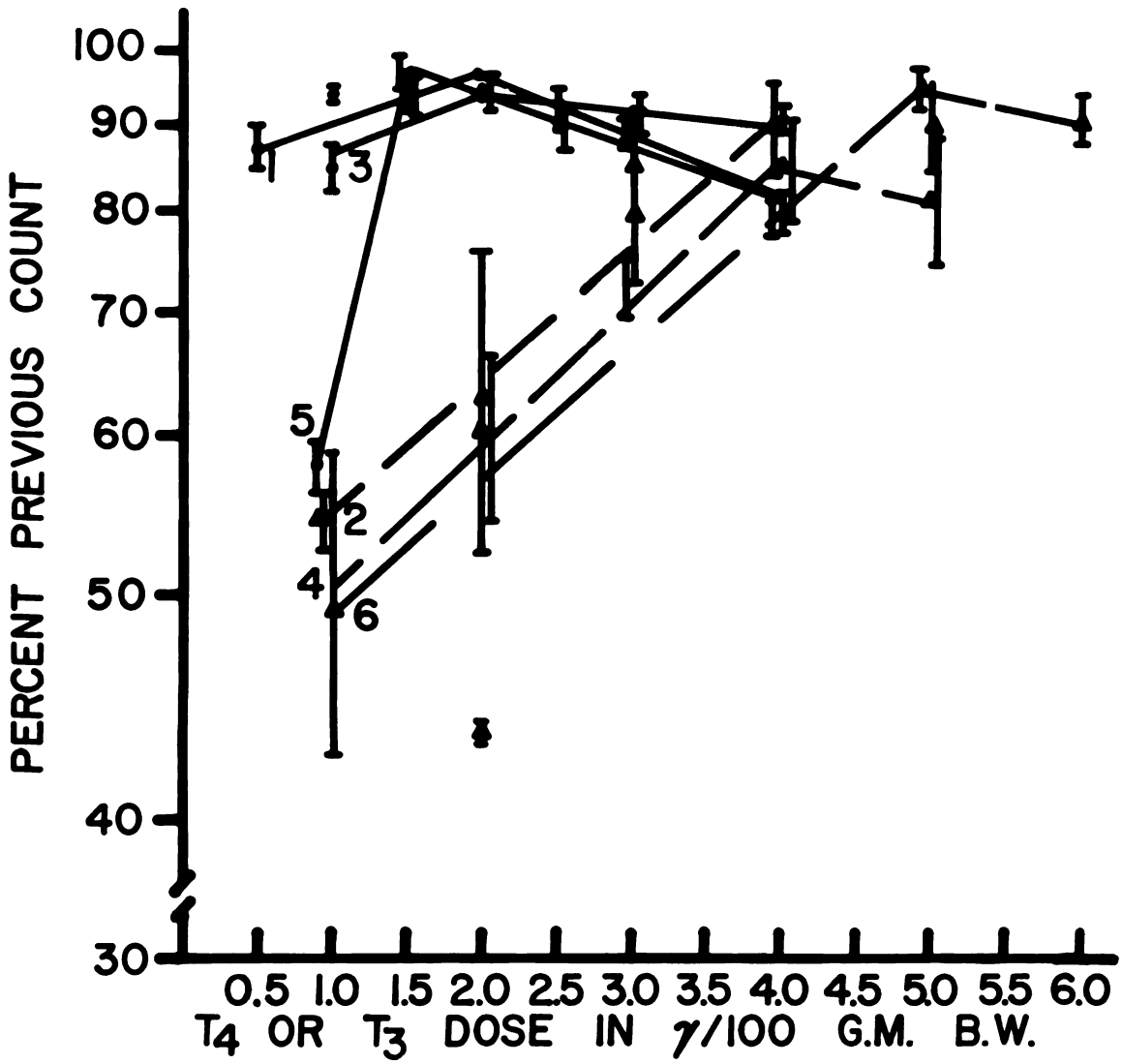
Table 1. Thyroid secretion rate in chickens by thyroxine and triiodothyronine substitution methods.

Group	No. of Birds	Age (weeks)	Thyroidal I^{131} Retention at TSR Estimation, Percent Previous Count	Estimated TSR µg/100 g b.w./day
T_4, normal	9	5.5	96.5 ± .67	2.00 ± .167
T_4, tapazole treated	7	2	90.5 ± 5.93	4.14 ± .34
T_3, normal	9	5.5	94.0 ± 2.11	2.17 ± .2762
T_3, tapazole treated	7	2	85.4 ± 6.0	3.86 ± .34

Mean ± standard error.

Estimated TSR values are significantly greater in tapazole-treated than in normal groups. (For T_4 $U = 1$, $P < .002$. For T_3 $U = 6$, $P = .02$.)

TSR differences between the two normal groups and between the two tapazole-treated groups are non significant ($U = 37.5$, $P > .05$ and $U = 20$, $P = .620$ respectively) by Mann-Whitney U test.



SUBSTITUTION OF:

(1) T_4 - NORMAL CHICKS

(2) T_4 - TAPAZOLE TREATED CHICKS

(3) T_3 - NORMAL CHICKS

(4) T_3 - TAPAZOLE TREATED CHICKS

(5) T_4 - NORMAL CHICKS, LOW IODINE DIET

(6) T_4 - TAPAZOLE TREATED CHICKS, LOW IODINE DIET

(AVERAGE CURVES)

Figure 2. Substitution of thyroxine or triiodothyronine in the normal or tapazole-treated chicks on diets adequate or deficient in iodine.

birds are still releasing labelled products from the thyroid at a rate equivalent to the normal one.

In the present study (Table 1, Figure 2) no significant difference was found in the thyroid suppressing effect of T_3 and T_4 as indicated by TSR values of the two normal or the two tapazole-treated groups. Per unit weight of the hormones, the potencies of T_3 and T_4 are not significantly different. On the iodine basis, however, T_3 is slightly more active. The end point for T_4 was 2.0 µg, equivalent to 1.31 ug of iodine. For T_3 it was 2.17 µg equivalent to 1.27 µg iodine. In view of the experimental variation to be expected in this type of comparison and the smallness of the difference in activity, whether on a weight or iodine basis, the entire thyroidal iodine output has been calculated as T_4 in subsequent experiments by the direct output method.

The results of different workers differ with regard to relative potencies of the two hormones in the chicken (Gilliland et al., Shallabarger, Newcomer, Newcomer and Barret, Mellen and Wentworth--already cited). Some workers report that T_3 is more potent than T_4. Others report the opposite and still others think both hormones are equally potent. In mammals T_3 is generally considered 4-6 times more potent than T_4. Recently, Bauman, Pipes and Turner (1965) observed that T_3 is 2.6 times more potent than T_4 by the substitution method in rats. Rat substitution assay in this laboratory shows T_3 to be 4 times as potent as T_4 per unit

weight and 4.6 times as potent as T_4 per unit of iodine (Reineke and Lorscheider unpublished).

Figure 2 also shows that when a given T_4 or T_3 dose representing estimated TSR, blocks maximally the I^{131} output from the thyroid, a further increment in the hormone dose was not able to hold that blocking level. Apparently some leakage of the iodine does occur, despite administration of sufficient hormone which should supposedly suppress completely the pituitary TSH secretion. Similar observations have been made in rats (Reineke and Singh, 1955) and in chickens fed thiouracil (Mellen and Wentworth, 1958).

Direct Output Method

Figure 3 shows typical TSR determination by the direct output method. There is a close agreement in the TSR of different groups of chickens as estimated by this method (Table 2). The statistical analysis showed no significant difference in TSR among birds of different ages. A trend for higher TSR is, however, noticed in week-old chickens. The highest TSR was observed in 4.5-week-old chickens, which could possibly be due to their higher thyroidal iodine content. This group was fed with the chick starter diet (Appendix G). All other groups were given either SR-1 or SR-2, supplemented with iodine (Appendix H). A study of the effect of three diets on body weight, thyroid weight and

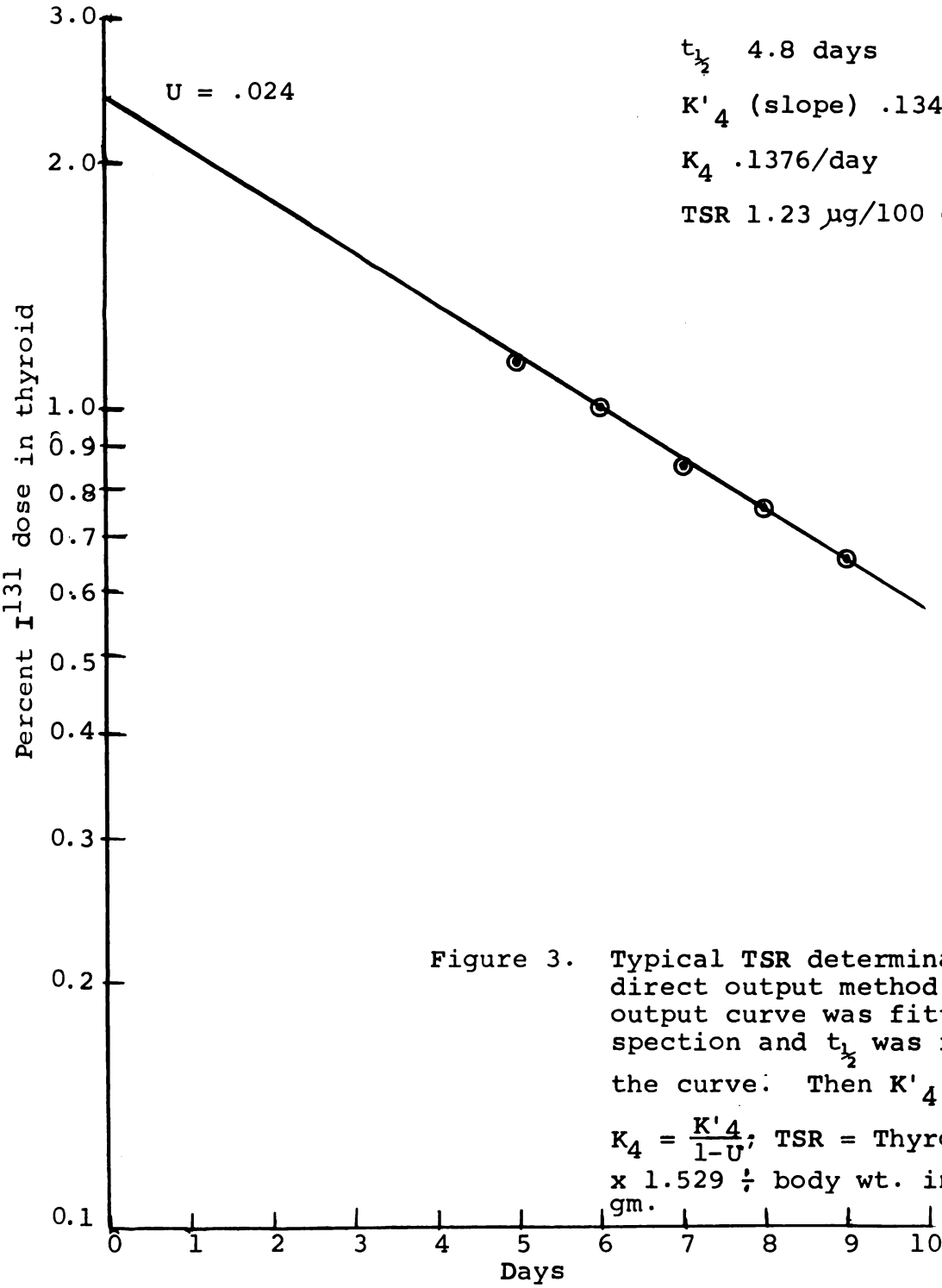


Figure 3. Typical TSR determination by direct output method. The output curve was fitted by inspection and $t_{1/2}$ was read from the curve. Then $K'_4 = \frac{0.693}{t_{1/2}}$; $K_4 = \frac{K'_4}{1-U}$; TSR = Thyroid I x K_4 x 1.529 ÷ body wt. in 100's of gm.

Table 2. Direct output data in chickens.

Age (Weeks)	No. of Chickens	Body Weight (gm)	Zero Time Percent Uptake U	K_4	Total Thyroidal Iodine	TSR/100 gm b.w./day
1	10	91.30 ± 2.375	.0375 ±.0223 (8)**	.2809 ±.037	2.67 ± .26	1.2005 ± .208
3	10	200.60 ± 10.47	.0802 ±.0108	.1365 ±.020	8.95 ± 1.414	.823 ± .118
4*	10*		.0628 ±.0049	.1282 ±.0126	13.98 ± 1.55	
4.5	9	424.78 ± 11.16	.0660 ±.0076 (10)**	.1112 ±.01 (10)**	34.06 ± 4.32	1.30 ± .153
9	10	1456.0 ± 32.94	.1518 ±.0188	.0493 ±.0088	216.56 ± 21.11	1.077 ± .20

Mean values ± standard error.

Differences of TSR/100 gm b.w. among different groups of chickens are non significant by one way analysis of variance ($F_{3.34} = 1.35$, $P > .05$).

*Average daily total TSR of this group, not adjusted for body weight equals 2.68 ± .2497.

**Number of observations included in the mean.

thyroidal iodine revealed no significant difference between thyroidal iodine of chickens fed chick starter diet and SR-2 supplemented with iodine.

The relationship between daily I^{131} output from the gland (K_4) and age of chickens is presented in Figure 4. K_4 decreases significantly in older birds. There is a strong correlation coefficient (r) = -.8351, $P < .005$ in chickens from 1-9 weeks of age. Figure 4 also shows a relationship between thyroidal iodine per unit body weight and age of chickens. This again has a strong correlation coefficient of -.8869, $P < .005$. It thus seems that as the birds grow older, decrease in output rate is balanced by an increase in thyroidal iodine content such that the thyroid secretion rate per unit body weight is little affected. This fact is further indicated by an inverse relationship between the K_4 and the thyroidal iodine per 100 gm body weight. This relationship is significant, with a correlation coefficient of -.8285, $P < .005$.

The thyroid secretion rate in the direct output method is finally estimated (Figure 3) through a series of calculations involving the above mentioned parameters. The relationship between TSR and thyroidal I^{131} release rate or uptake are biased because these parameters are related. Nevertheless, the K'_4 and the U (Zero time percent uptake) being independent observations, their relationship was

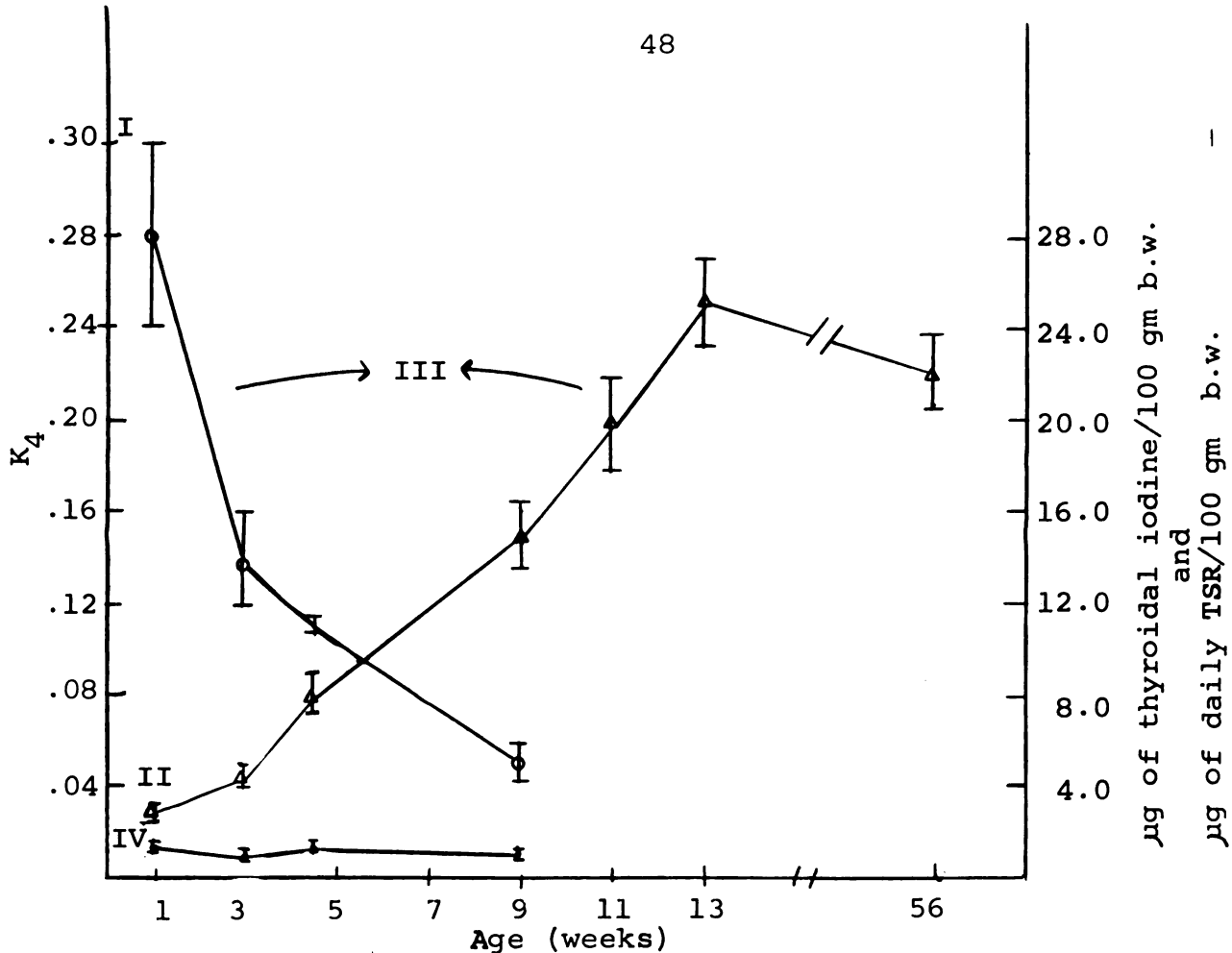


Figure 4. Relationship between (I) K_4 and age of chicks. (II) Thyroidal Iodine/100 gm b.w. and age of chicks. (III) K_4 and thyroidal iodine/100 gm b.w. of chicks.

This graph shows mean values ± standard errors. Regression and correlation coefficients for each relationship are given below.

Curve (IV) shows TSR/100 gm b.w./day by the direct output method.

I	II	III
K_4 vs. age	Thyroidal Iodine /100 gm b.w. (up to 13 wks.) vs. age	K_4 vs. thyroidal iodine/100 gm b.w. (up to 9 wks.)
$\log Y_X = \log a + b \log X$	$Y_X = a + bx$	$\log Y_X = \log a + b \log x$
b = -.7691	b = 1.8522	b = -.8384
a = -.5620	a = -.2873	a = -.0438
P <.005	P <.005	P <.005
r = -.8351	r = .8869	r = -.8285

determined in the growing chicks up to 9 weeks of age. They have a correlation coefficient of -.6719 and $P < .005$ (Figure 5).

The direct output method does not seem to have been previously employed in chickens. This method has also been little used in other animals and thus information about its various parameters is lacking. The knowledge available about the thyroidal I^{131} uptake has usually been obtained for a particular time after I^{131} administration. Under these situations it is largely agreed that in normal animals no simple relationship exists between the TSR and the I^{131} uptake or release rate (Turner et al., Lodge et al., Flamboe and Reineke, already cited). In sheep however, a low negative correlation between the TSR and the zero time percent uptake was found by Hoersch, Henderson, Reineke and Henneman (1961). The thyroidal I^{131} uptake and release rates are affected by a wide variety of factors such as size, weight, colloidal content, dietary iodine content, kidney function, pregnancy and lactation, etc., etc. It is thus not surprising that many workers could not observe relationships between other parameters and the TSR.

In this study on chickens, daily I^{131} output rate has been seen to be significantly higher in the younger birds and the thyroidal iodine per unit body weight significantly higher in older birds. It therefore appears that as

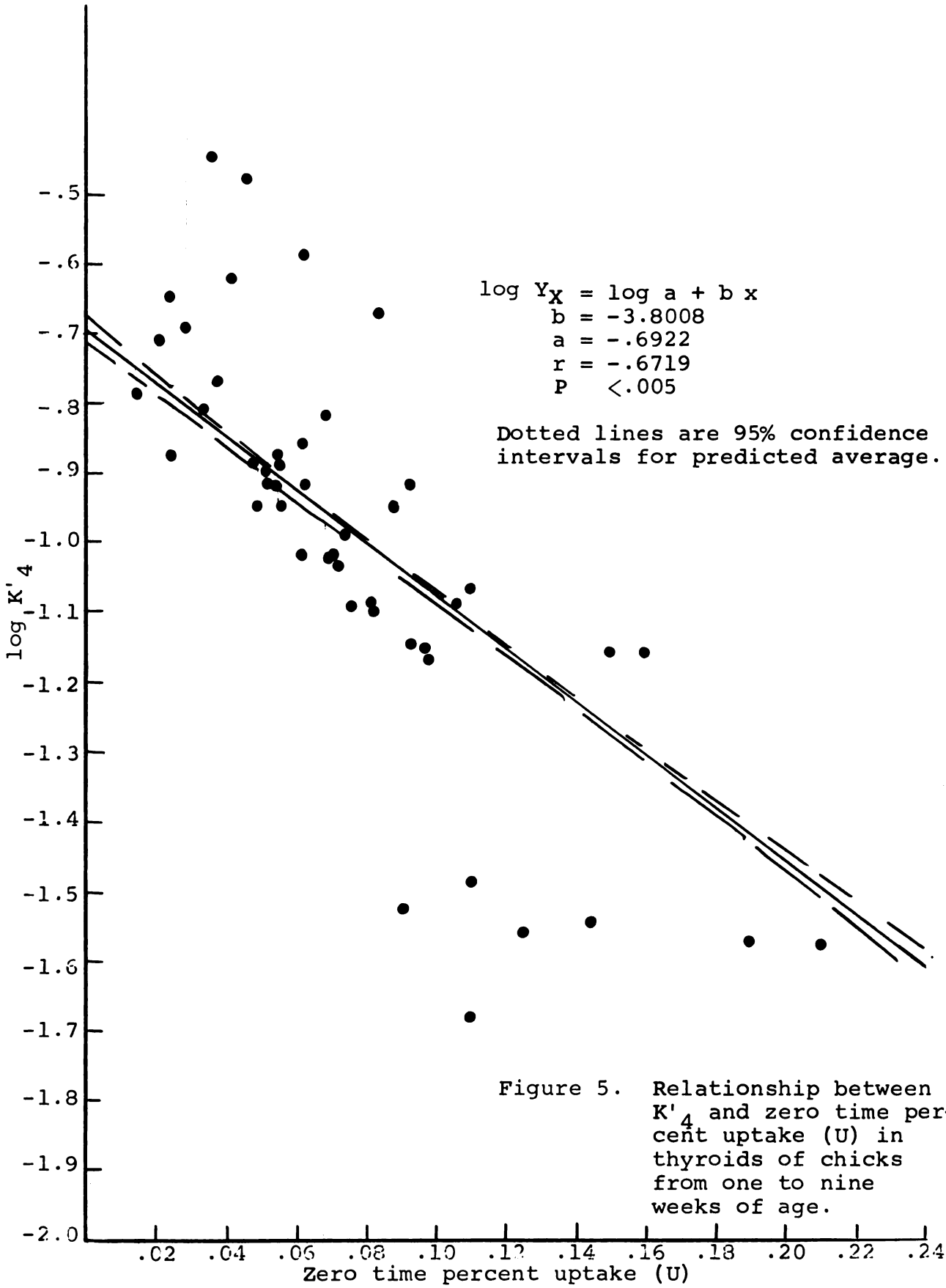


Figure 5. Relationship between K'_4 and zero time percent uptake (U) in thyroids of chicks from one to nine weeks of age.

the bird grows, its thyroid weight increases, the gland accumulates increased I^{127} which is associated with a decrease in thyroidal iodine release rate. It is for this reason that the researchers working with mature or nearly mature chickens find an almost flat output curve. For this reason some workers have used a goitrogen to accelerate the release rate.

It is interesting to notice a strong relationship between ug of total thyroidal iodine and gram body weight of growing chicks from 1 to 13 weeks of age (Figure 6). On a log-log plot the regression of thyroidal iodine upon body weight is nearly a perfect fit with a correlation coefficient of .97845, $P < .005$. This relationship does not hold for mature chickens. In adult birds, including chickens, bobwhite quail and coturnix, the total thyroidal iodine per unit body weight has been correlated with the thyroid weight per unit body weight (Figure 7). These two measures are again highly related with $r = .9416$, $P < .005$. Both these relationships are close enough to allow a good estimation of the iodine content in thyroids from body weights of growing chicks and from thyroid weights of adult birds, provided the dietary content of iodine is adequately controlled. In growing chicks it may also be possible to estimate their thyroid secretion rate by the direct output method without killing them for recovery of their thyroids and iodine determination.

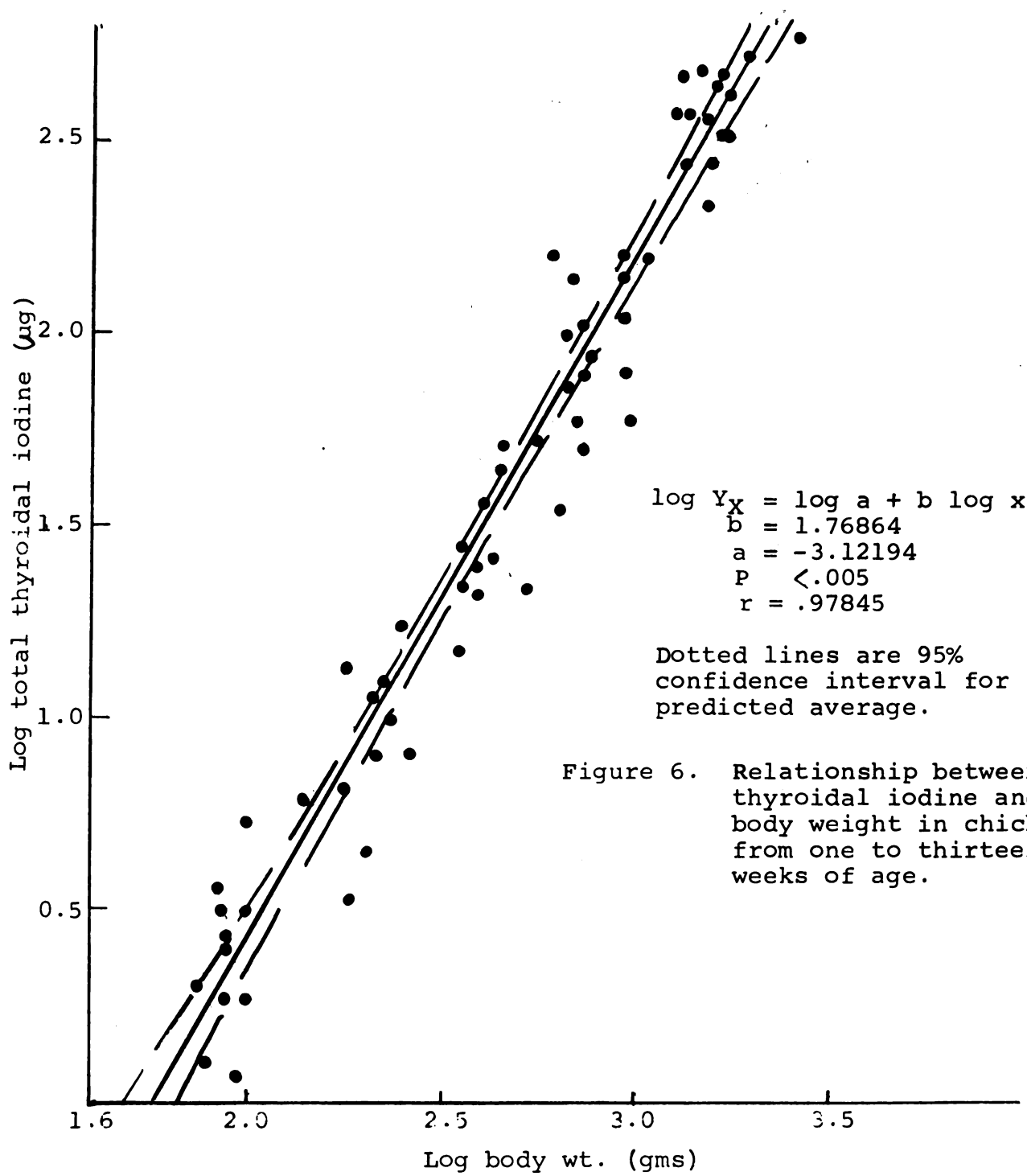


Figure 6. Relationship between thyroidal iodine and body weight in chicks from one to thirteen weeks of age.

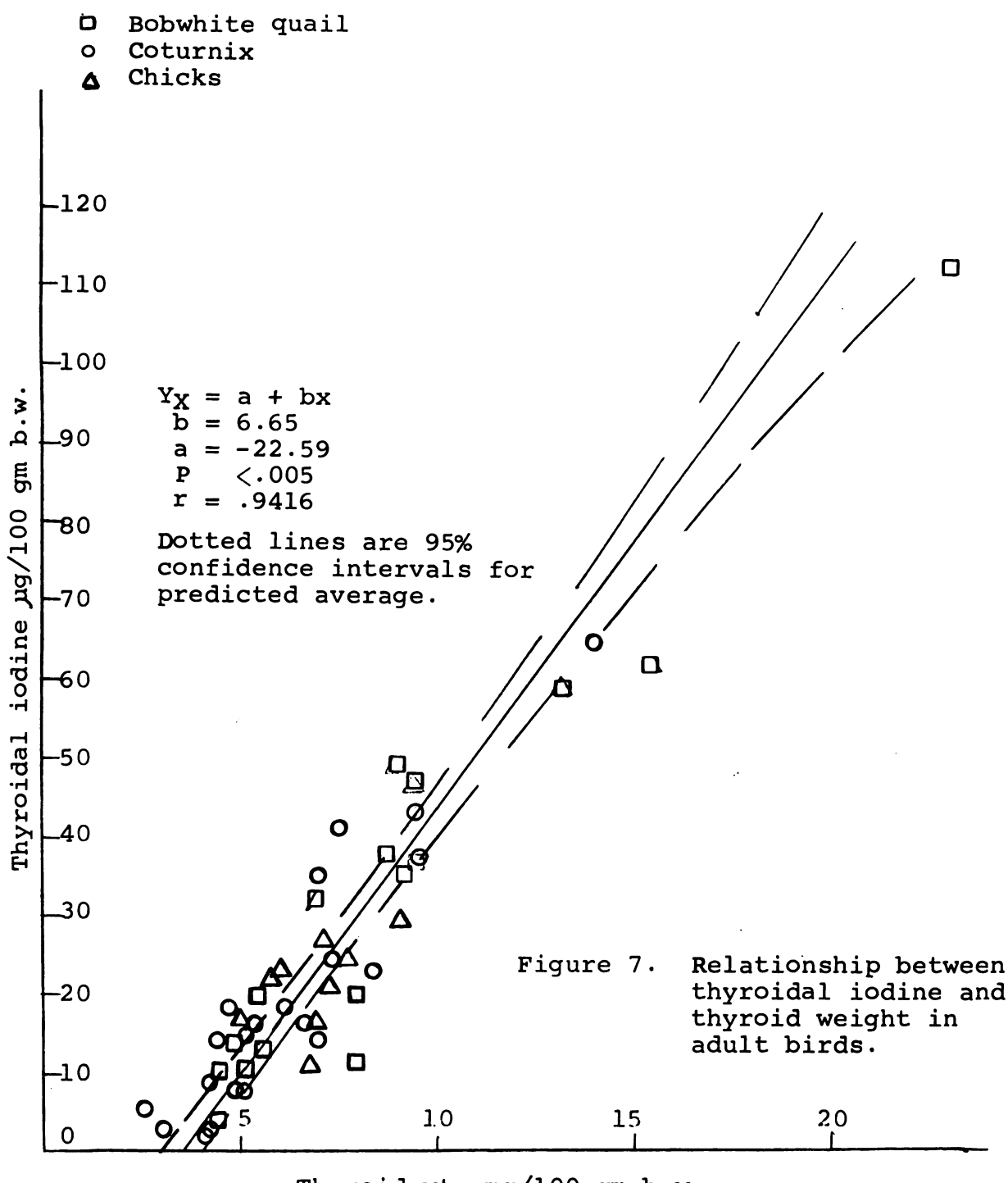


Figure 7. Relationship between thyroidal iodine and thyroid weight in adult birds.

Thyroid Hormone Degradation Method

Thyroid secretion rates of 7-week normal chicks, 7-week goitrogen-treated chicks, 56-week normal chicks, 56 to 58-week bobwhite quail and 10-week coturnix, as determined by the thyroxine degradation method averaged 2.03, 1.59, 1.02, 2.49 and 2.78 μg/100 g/day, respectively. It shall be seen from Figure 8 that the TSR in this method is calculated from three parameters: the biological half life ($t_{\frac{1}{2}}$), the thyroxine distribution space (TDS) and protein-bound iodine (PBI). The values of these parameters influence the estimated TSR. The $t_{\frac{1}{2}}$, TDS and PBI were also determined for all birds in the T_3 degradation experiment but the TSR was not calculated as the exact information with regard to plasma-protein binding of T_3 and its contribution to the PBI of chickens is not available in the literature. The thyroid hormone degradation method has not been previously used for estimation of TSR in birds. There is, thus, little information with which to compare the results obtained in this study.

Biological Half Lives ($t_{\frac{1}{2}}$)

The biological half lives of T_4 and T_3 are not significantly different in bobwhite and Japanese quail (Table 4). The apparent differences are due to an interaction

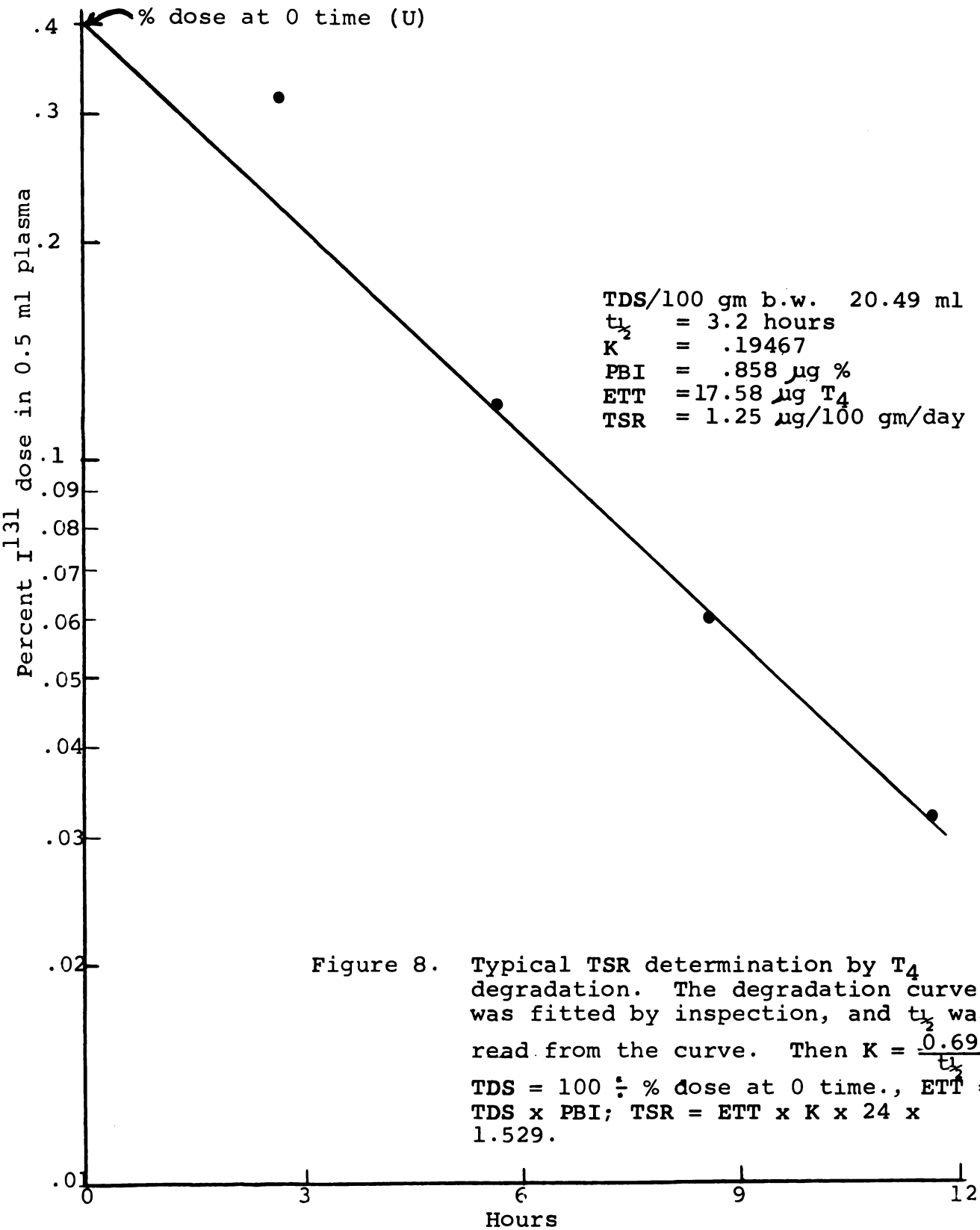


Figure 8. Typical TSR determination by T_4 degradation. The degradation curve was fitted by inspection, and $t_{1/2}$ was read from the curve. Then $K = \frac{0.693}{t_{1/2}}$; TDS = 100 ÷ % dose at 0 time., ETT = TDS x PBI; TSR = ETT x K x 24 x 1.529.

between the two hormones and the two breeds of quail. In the case of chickens, only the $t_{\frac{1}{2}}$ of T_3 in 56-week normal chickens was significantly greater than in all other groups of chickens treated by both T_3 and T_4 (Table 3). The interaction between the three groups of chickens and the two hormones is significant.

It is interesting to note that the biological half lives of T_4 and T_3 are relatively much shorter in birds than in man or other mammals (see literature review). Heninger and Newcomer (1964) reported mean half lives of 4.9 and 3.9 hours of T_4 and T_3 respectively in the cardiac tissue of chickens. These values are close to the $t_{\frac{1}{2}}$ of T_4 and T_3 in the chicken plasma observed in this study. McFarland, Yousaf and Wilson (1964) reported fractional turnover rates of T_4 in coturnix, which when expressed as $t_{\frac{1}{2}}$ ranged approximately from 17 to 27 hours at 70° to 90°F. These values are much higher. A further check of their report revealed that they had taken cardiac blood samples, which have been observed in the present study, to cause a previously unnoticed reaction resulting in an increased radioactivity in the blood and an overestimation of $t_{\frac{1}{2}}$ values.

Thyroid Hormone Distribution Space (TDS)

The distribution spaces of T_3 were significantly higher than of T_4 in both chickens and quail (Tables 3 and 4).

Table 3. Statistical analysis of turnover data of thyroxine and triiodothyronine in chickens.

Two way analysis of variance with 6 replicates.

Source	Distribution space ml. per 100 gm. b.w.			$t_{\frac{1}{2}}$ (Hours)		
	d.f.	mean ss	F	d.f.	mean ss	F
Chickens	2	149.07	2.12	2	40.49	26.17***
T_4 and T_3	1	14697.93	209.55***	1	49.47	31.98***
Interaction	2	205.65	2.93	2	22.345	14.44***
Error	30	70.14		30	1.547	

New Multiple Range Test*

	E	F	D	A	B	C		F	E	C	D	A	B
Means	63.87	61.35	60.46	29.39	20.53	14.52		9.35	3.86	3.86	3.76	3.23	2.85

Table of observed minus adjusted means for $t_{\frac{1}{2}}$ values showing interaction between the three groups of chickens and the two thyroid hormones.

Hormones	7 wks. Normal	7 wks. Goitrogen Treated	56 wks. Normal
T_4	.905	.664	-1.569
T_3	-.905	-.664	1.569

7 wks. normal, 7 wks. goitrogen treated and 56 wks. normal groups of chickens from T_4 degradation experiment are respectively termed as A, B and C. Similar groups from T_3 degradation experiment are termed D, E and F. Each group contained 6 birds.

*Significant at 0.05 level.

***Significant at 0.005 level.

Under scored non significant.

Table 4. Statistical analysis of turnover data of thyroxine and triiodothyronine in quail.

Two way analysis of variance with 9 replicates.

Source	Distribution space ml. per 100 gm. b.w.			$t_{1/2}$ (hours)		
	d.f.	mean ss	F	d.f.	mean ss	F
Quail	1	.4482	64.49***	1	.04656	3.033
T_4 and T_3	1	.7606	109.43***	1	.02083	1.357
Interaction	1	.0501	7.21**	1	.14350	9.348***
Error	32	.00695		32	.01535	

New Multiple Range Test* (Distribution Space)

	D	C	B	A
Log. means	1.96276	1.81428	1.74673	1.44891

Table of observed minus adjusted means showing interaction between two groups of quail and the two thyroid hormones.

Hormones	Distribution Space		$t_{1/2}$	
	Bobwhite	Coturnix	Bobwhite	Coturnix
T_4	-.0373	.0373	-.0631	.0631
T_3	.0373	-.0373	.0631	-.0631

Variance homogeneity was checked by Bartlett's test. H_o being rejected, log transformation was done on the data and variance homogeneity checked again. Two Bobwhite samples from T_4 and one from T_3 groups, whose PBI values were off the normal range were eliminated to make equal numbers.

Groups A and B are Bobwhite and Coturnix in T_4 degradation experiment. Groups C and D are Bobwhite and Coturnix in T_3 degradation experiment respectively.

*Significant at 0.05 level.

**Significant at 0.025 level.

***Significant at 0.005 level.

Among the three groups of chickens treated by T_3, there were no significant differences in their distribution spaces, but in the case of T_4, the distribution spaces in 7-week normal chicks were significantly higher than in 56-week normal chicks. The TDS of both T_4 and T_3 in coturnix were significantly higher than those in bobwhite quail. A significant interaction existed between the quail and the thyroid hormones.

The decrease of TDS of T_4 in older chicks could be an age effect. Gregerman et al. (1962) reported an age decrease of TDS in man after decade 6 and he suggested that the decrease of metabolic mass with age could influence the distribution space. Contrarily, Oddie, Meade and Fisher (1966) observed that the distribution space is independent of age and height in man. The above mentioned workers also reported a positive correlation between the distribution space and the body weight in man. In the case of 6- to 7-week old chickens regression of the thyroxine distribution space on body weight was not found to be significant ($F\ 1, 13 = 3.28$, $P > .05$). The relationship between the triiodothyronine distribution space and body weight of 7-week normal and goitrogen-treated and 56-week normal chickens was tested by the Spearman rank correlation coefficient. This showed a significant positive correlation with values of $rs = .9023$ and $P < .02$ (two tailed).

Following administration of the labelled hormones, the plasma samples taken after 3 to 12 hours contained half or nearly half the percent radioactivity for I^{131}-T_3 as for I^{131}-T_4. This indicates that T_3 disappears from the plasma at a faster rate than does T_4. Hutchins and Newcomer (1966) also observed that T_3 was metabolised and excreted at a greater rate than T_4 in chickens. Further, Flock, Owen and Paris (1966) reported that T_3 in rats was deiodinated and conjugated at a faster rate than T_4.

In an in vitro experiment on the uptake of T_4 and T_3 avian erythrocytes, it was observed that the red blood corpuscles took up 1.11 percent of I^{131}-T_4 as compared to 4.71 percent of I^{131}-T_3 after 2 hours incubation of the chicken blood mixed with I^{131} labelled hormones. Qualitatively similar results regarding the differential uptake of the two hormones by the avian RBCs were reported by Heninger (1962), although percent uptake of the two hormones varied in his experiments.

Rapid deiodination, metabolism and relatively larger uptake of T_3 by RBCs may account for higher TDS for T_3 than for T_4. This view is further supported by the observation that the triiodothyronine distribution spaces in young sexually immature and old sexually mature chickens are not significantly different (Table 3). Sexually mature males have a higher number of erythrocytes and the androgens, are

believed to be responsible for the difference (Sturkie, 1965). Thus, more RBCs in older birds take up more I^{131}-T_3, which may result in a decreased radioactivity in the plasma and an increased calculated TDS. The coturnix have higher T_4 and T_3 distribution spaces than chickens and bobwhite quail. This could possibly be due to a relatively smaller size, or a species characteristic of coturnix. The $t_{\frac{1}{2}}$ in T_4 and T_3 turnover of this quail was, however, not significantly different from that of other birds.

Increased Plasma Radioactivity Under Stress

During the course of plasma sampling it was noticed that in some birds their 12-hour samples (in some cases earlier samples) contained higher radioactivity than some of their earlier samples (Table 5). This kind of increased radioactivity was seen (1) more in I^{131}-T_4 than I^{131}-T_3 turnover experiments, (2) more in coturnix than in bobwhite quail, (3) more in goitrogen-treated and normal older birds than in the normal younger birds, (4) the discharge was especially well marked in the samples taken by heart puncture, (5) the discharge also seemed to be related to the amount of the blood drawn from the bird. Presumably, the increased radioactivity resulted from a stress to the birds at this stage. The discharge did not seem to have any relationship to the blood volume, as the heavier chickens showed more than the lighter ones.

Table 5. Percent increase of radioactivity in blood as calculated in the 12-hour sample taken under stress.

	Chickens			Quail	
	Normal 6* (7)**	Goitrogen treated 6* (7)**	Normal 6* (56)**	Bobwhite 11* (56-68)**	Coturnix 9* (10)**
I^{131}-T_4	8.72 ±4.86	23.08 ± 6.86	25.35 ±10.34	33.23 ± 7.17	134.94 ± 19.34
I^{131}-T_3	3.25 ±1.76	6.38 ± 2.46	4.87 ± 3.31	24.68 ± 8.03	67.93 ± 18.29

Mean ± standard error.

*Number of birds in group.

**Age of birds in weeks.

In studies on rats, dogs and men, Taurog et al. (1951, 1952) and Albert and Keating (1949) observed that the thyroxine concentrates in the liver even when it is administered intravenously. They thought that T_4 probably undergoes an enterohepatic circulation. Recently, Gorman et al. (1966) isolated and perfused livers of rats at 1 to 20-hour intervals following administration of the thyroid hormones. They observed that the unchanged T_4 was released to the perfusing blood until an equilibrium was reached. They also reported that the livers of rats given labelled T_3 released only a small amount of the hormone to the perfusing blood. These differences between T_3 and T_4 together with a lower affinity of T_3 for the plasma proteins and a faster metabolism of T_3 may account for higher discharge seen in the 12-hour samples in the T_4 than in the T_3 degradation experiment.

Protein-Bound Iodine (PBI)

The results of PBI analyses are given in Table 6. There was little overall difference in PBI levels of different groups of chickens and coturnix. The bobwhite quail, however, showed higher PBI, both in analyses of individual samples, as well as in the pooled plasma. The author is not aware of any report already published about PBI of quail. In the chicken, the results obtained in this study are in agreement with those reported by Bumgardner and Shaffner (1957)

Table 6. Protein bound iodine (µg per 100 ml. plasma) in chickens and quail.

Birds	Number of Birds	Age (wks.)	Iodine Content of Diet	PBI
Chickens	43	5-7	Adequate	1.1226 ± .0367
Chickens	18	5-6	Deficient	1.0135 ± .0483
Chickens	11	56	Adequate	1.2840 ± .0975
Bobwhite quail	18 10*	56-68	Adequate	1.7572 ± .0637 1.4770*
Japanese quail	17 10*	10	Adequate	1.2603 ± .1320 .9344*

Mean ± standard error.

*PBI of pooled plasma from 10 other quail.

and Mellen and Hardy (1957). The values reported by Rosenberg et al. (1964) were relatively lower. The chickens on the diet deficient in iodine had slightly lower levels of PBI than those on adequate iodine diet. Similar non-significant differences were noted by Rosenberg et al. (1964).

Lower levels of PBI in birds are in marked contrast to the situation in mammals whose average PBIs range from 3-6 µg percent. The absence of a specific thyroxine-binding alpha-globulin in avian blood may contribute to this difference. Further, short biological half lives of the thyroid hormones in birds as observed in the turnover studies and an insignificant increase of M.R. following T_4 injections in birds, may be associated with lower levels of PBI, as the latter is an estimation of the thyroid hormones in the blood.

Effect of Iodine Intake on Thyroid Function

The effects of dietary content of iodine on body weight, thyroid weight, thyroidal iodine and the estimated TSR by different methods are given in Tables 7 and 8. A significant decrease of body weight in the iodine-deficient group shows how a low iodine intake can impair thyroid function which in turn depresses growth gains in chickens. Excessive amounts of this mineral produce iodine toxicity. This is associated with cessation of egg production and

Table 7. Effect of dietary content of iodine on body weight, thyroid weight (mg/100 gm b.w.) and thyroidal iodine (µg/100 gm b.w.) of 7 week-old chickens.

One way analysis of variance

Source	Body weight d.f.	mean ss	F	Thyroid weight d.f.	mean ss	F
Treatment	2	19515.03		2	66.86	
Error	27	3699.85	5.274*	27	6.14	10.89***

New Multiple Range Test*

	A	B	C	C	A	B
Means	721.40	702.80	637.30	11.32	7.23	6.53

One way analysis of variance

Source	Thyroidal Iodine d.f.	mean ss	F
Treatment	2	385.26	19.94***
Error	26	19.32	

New Multiple Range Test*

A	B	C
12.68	8.56	0.47

Treatments A, B and C are respectively the diets chick starter, SR_2 with 1.3 µg/gm feed, and SR_2 deficient in iodine.

*Significant at .05 level.

***Significant at .005 level.

Underscored non significant.

10 chicks in all the groups, 9 in thyroidal iodine with B treatment group.

Table 8. Effect of dietary iodine content on various parameters of thyroid secretion rate determined by different methods in chickens.

Parameter	Diet Adequate in Iodine	Diet Deficient in Iodine	Level of Statistical Significance by Mann-Whitney U Test	
			U	P
U (Zero time % Maximum Uptake)	0.0660 ± .0076 10(4.5)	0.0926 ± .0068 10(4.5)	20.5	<.05
K_4	0.112 ± .01 10(4.5)	0.1801 ± .019 10(4.5)	14	<.02
Thyroidal Iodine µg per 100 gm b.w.	7.90 ± .87 9(4.5)	1.74 ± .043 10(4.5)	0	<.002
TDS_{ml}/100 gm b.w.	33.88 ± 3.075 9(6)	10.78 ± 1.068 8(5.5)	0	<.002
$t_{\frac{1}{2}}$ in plasma (Hours)	3.44 ± .20 9(6)	3.86 ± .28 8(5.5)	27	>.05
PBI µg %	0.90 ± .063 9 (6)	1.12 ± .09 8(5.5)	17	>.05
ETT µgI*	0.30906 ± .037 9(6)	0.12092 ± .0148 8(5.5)	1	<.002

TSR by T_4 Substitution on normal birds	2.00 ± .167 9(5.5)	1.66 ± .083 10(4)	24	>.05
TSR by T_4 Substitution on tapazole treated birds	4.14 ± .34 7(2)	4.89 ± .111 9(4)	16	>.05
TSR by direct output	1.30 ± .153 9(4.5)	0.46 ± .051 10(4.5)	0	<.002
TSR by T_4 degradation	2.03 ± .15 9(6)	0.73 ± .087 8(5.5)	0	<.002

Mean values ± standard error.

Figures below the values within and without parenthesis are respectively the age (in weeks) and number of chickens.

TSR is in µg/100 g b.w./day.

*Multiply with T_4 iodine equivalent (1.529) to convert into µg T_4.

decreased fertility (Perdomo, 1966). It is therefore recommended that poultry rations should be supplemented by an adequate amount of iodine. The thyroidal content is significantly increased in the chicken fed adequate iodine diet. This indicates the capacity for iodine retention in the chick thyroid. Rosenberg et al. (1964) reported that in thyroids of chickens on iodide supplemented diet, the rate of trapping was fivefold higher than in rats. The differences in iodine content of the thyroid due to differences in dietary iodine intake also place limitations on the relationships between the thyroidal iodine and body weight in young chicks and thyroidal iodine and the thyroid weight of adult birds. These relationships hold good under controlled dietary iodine contents. The zero time uptake of I^{131} and K_4 are significantly increased by inadequate intake of iodine. This is possible because iodine deficiency is associated with increased TSH secretion, which in turn stimulates the thyroid gland whereby the U uptake and the daily output rate are both increased. The thyroxine distribution space is significantly decreased with deficient iodine intake. The TDS reflects the calculations of ETT which is also accordingly affected. Iodine content of the diet does not seem to alter PBI and $t_{\frac{1}{2}}$ of thyroxine in the plasma.

The thyroid secretion rates determined by direct output and T_4 degradation methods are significantly increased

when the diet is adequate in iodine, but there was no significant difference due to dietary iodine intake in TSR estimated by the T_4 substitution method in normal and tapazole-treated birds. The results of the direct output and substitution methods in chickens are in keeping with those in rats reported by Reineke (1965).

Effects of Thyroxine on Growth, Feather and Comb Development

Effects of thyroxine and tapazole on growth of chickens is shown in Figure 9. Groups 2, 3, 4, and 5 receiving respectively 0.5, 2.0, 3.0 and 4.0 µg of T_4 per 100 g b.w./day gained more weight than the normal control group. Growth gains in T_4-treated groups were in the order of the doses given. The growth of birds in Group 6, treated with tapazole only (hypothyroid control, T_x) was most retarded. Groups 7, 8, 9, and 10 receiving both tapazole and replacement therapy at the rates of 1.0, 2.0, 3.0, and 4.0 µg T_4/100 g b.w./day, respectively, were heavier than the normal and the hypothyroid controls. Groups 8 and 9 receiving 2.0 and 3.0 µg T_4, however, gained more weight than group 10 receiving 4.0 µg T_4.

In another series of experiments, not included in Figure 9, the tapazole-fed birds given daily injections of 3.0 and 5.0 µg of T_4/100 g b.w. showed relatively less growth than those receiving 2.0 µg T_4/100 g b.w. Moreover,

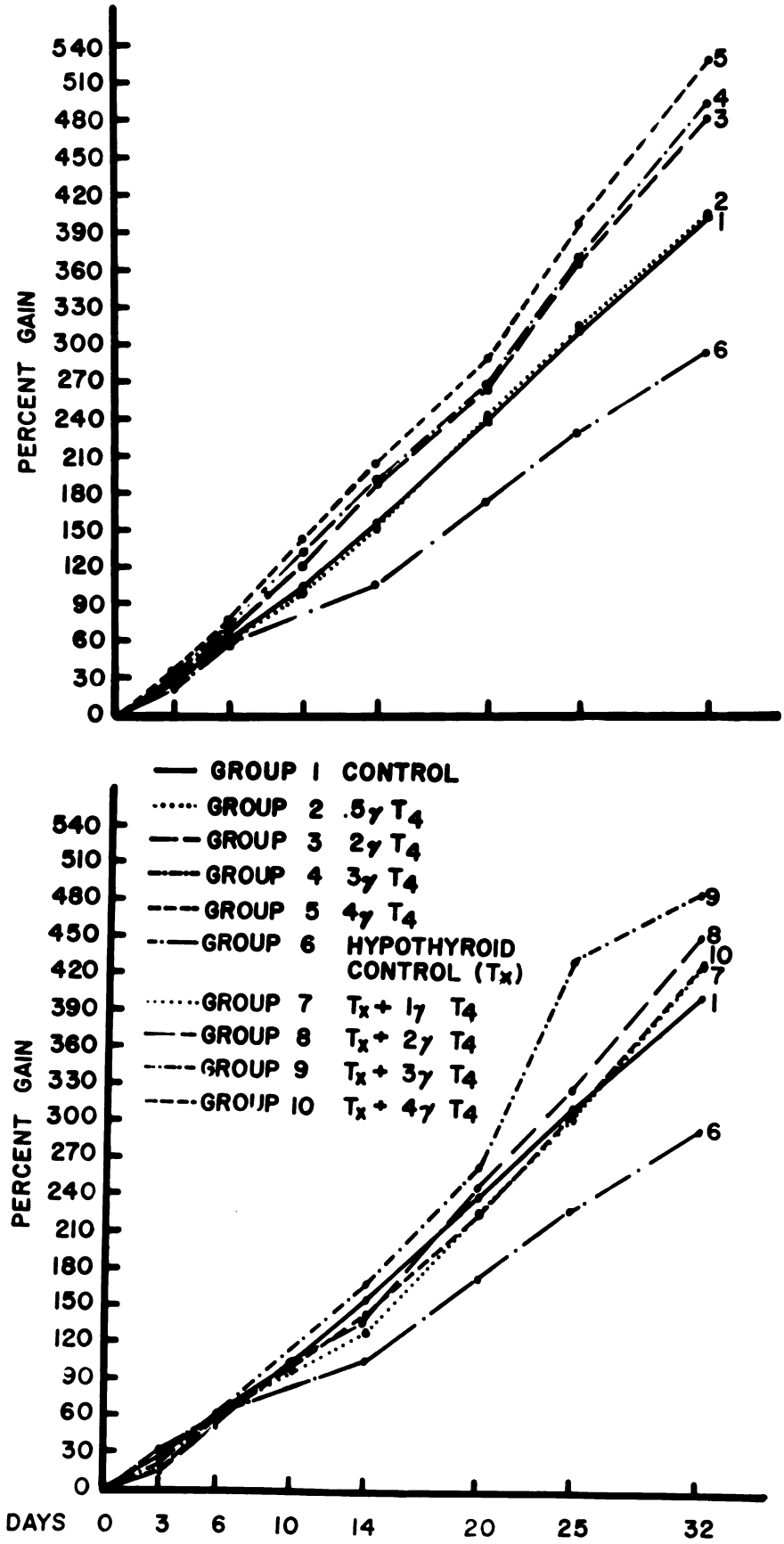


Figure 9. Effects of thyroxine and tapazole on growth of chickens.

in experiments on determination of M.R. growth of the birds receiving a daily dose of 6.0 µg T_4 or T_3 per 100 g b.w. for 26 days was depressed as compared to that of controls.

These observations indicate that thyroxine in small doses improves growth of chickens, but when administered beyond physiological doses, depresses growth rate. T_4 in toxic doses accelerates catabolic processes, and then the body weight is reduced. The observations described above also indicate that higher doses of T_4 administered to the tapazole-treated birds retarded growth rate more than when given to the normal birds. Although no explanation is readily available for such action of T_4, some unknown interaction may be possible between higher doses of the hormone and the goitrogen on a line similar to the one demonstrated by Seller and Schonbaum (1965) involving a goitrogen potentiating effect of small doses of T_4 and propylthiouracil.

The effect of thyroxine and tapazole on growth of wing and tail feathers and comb growth is shown in Figure 10. The hypothyroid group showed retarded growth of feather and comb. The feathers of the birds from this group looked fringed and lacked barbules. In all other groups to whom T_4 was given, either additionally or as a replacement therapy, the comb and feather measurements were normal or nearly normal. The size and shape of feathers is influenced by the gonadal and thyroidal hormones (Sturkie, 1965). Snedecor

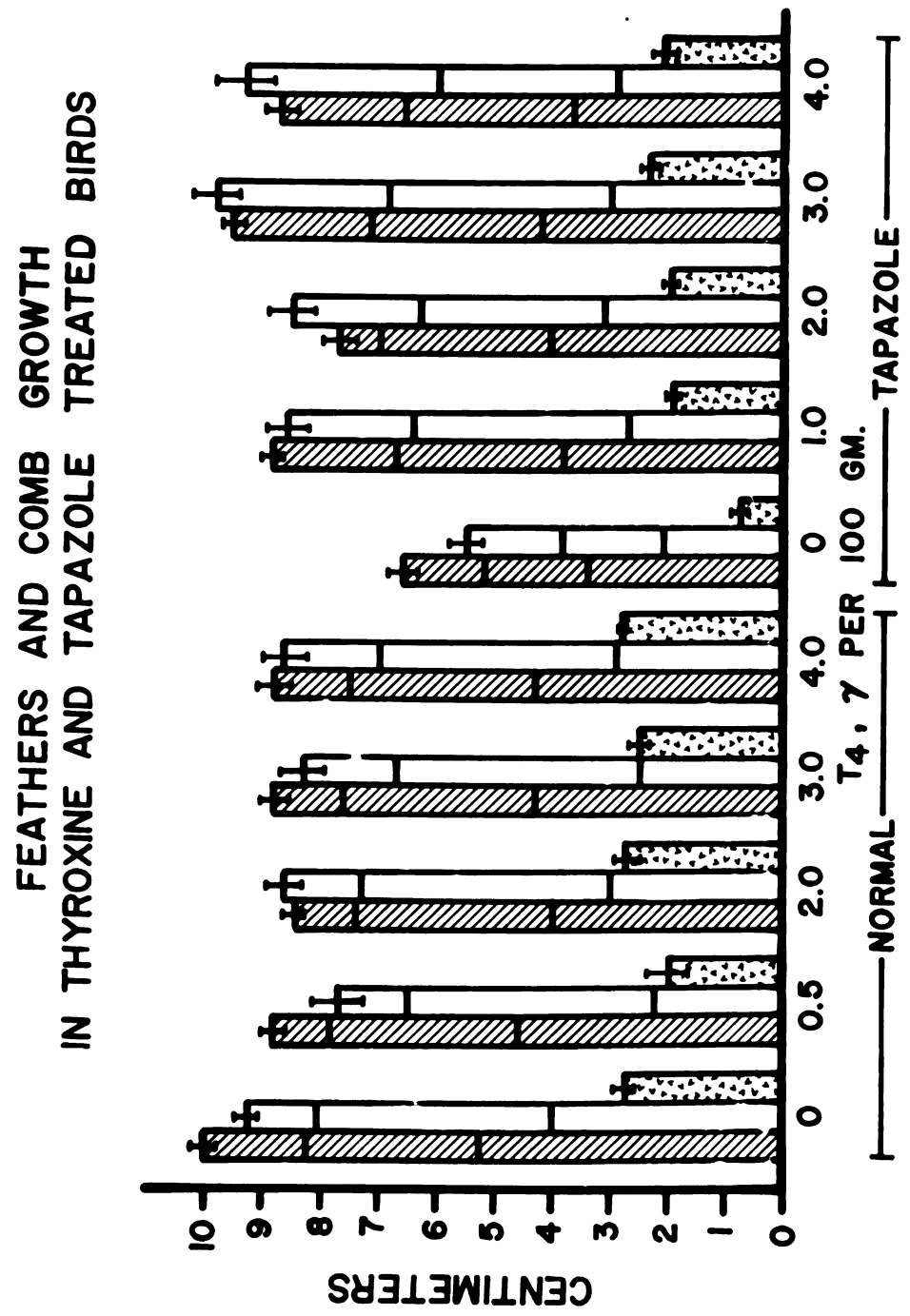


Figure 10. Influence of thyroxine and/or tapazole on feather and comb growth.

and Camyre (1966) have shown an interaction of androgen and the thyroid involving comb growth.

The thyroid hormones are necessary for growth and development of most tissues, such as special differentiation and maturation of epiphysial centres of ossification. The thyroid's stimulus to the growth may be due to (1) protein synthesis and nitrogen retention, (2) hastening of certain enzyme reactions by acting as chelating agent for some elements inhibitory to those enzymes, (3) increaing membrane permeability. T_4 increases peripheral utilization of glucose and (4) the thyroid hormone stimulates release of growth hormone from anterior pituitary or it is also believed that the growth hormone exerts its full effect in the presence of T_4.

The presence of a growth hormone or its exact function in chickens is uncertain. However, it seems clear that the thyroid hormone plays an important role in growth of the chicken.

Metabolic Rate (M.R.)

Table 9, Figure 11 and Appendices K and L show effects of the thyroid hormones on metabolic rate in chickens. A small increase in M.R. following administration of thyroxine, triiodothyronine or a combination of thyroxine and triiodothyronine lasts only for a short duration of 2-3

Table 9. Results of one way analyses of variance on M.R. in chickens.

	1 Hour after single injection of 4.0 µg/100 gm b.w.			2 Hours after single injection of 4.0 µg/100 gm b.w.		
Source	d.f.	mean ss	F	d.f.	mean ss	F
Among	2	104225.19	2.178*	2	267191	3.45**
Within	20	47847.95		19	77421	
	7 Hours after single injection of 4.0 µg/100 gm b.w.			2 Hours after daily administration of 6.0 µg/ 100 gm b.w. for 15 days.		
Source	d.f.	mean ss	F	d.f.	mean ss	F
Among	2	46262.33	1.70*	3	15714.22	0.38*
Within	18	27065.23		18	40803.91	
	3 Hours after daily administration of 6.0 µg/ 100 gm b.w. for 14 days.			24 Hours after daily administration of 6.0 µg/ 100 gm b.w. for 7 to 11 days.		
Source	d.f.	mean ss	F	d.f.	mean ss	F
Among	3	12537.22	0.25*	3	364367	6.4***
Within	17	49174.83		39	57003	

New Multiple Range Test on M.R. determined after 24 hours

	T_4	Control	T_3	$T_3 + T_4$
Means	1905	1851	1583	1546

*Or underscored = non significant at .05 level.

**Close to significance at 0.005 level.

***Significant at .005 level.

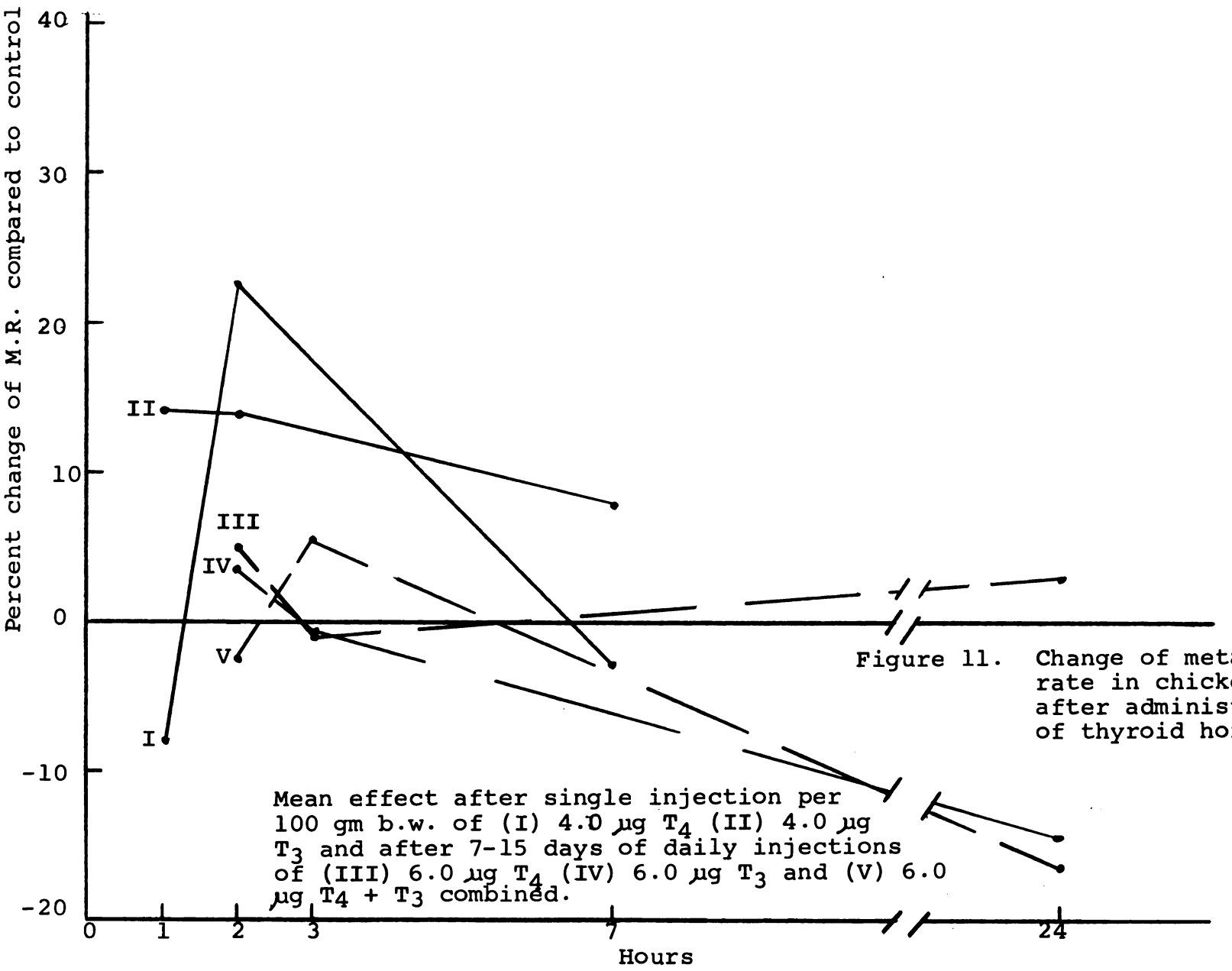


Figure 11. Change of metabolic rate in chickens after administration of thyroid hormones.

hours. The maximum effect was produced by T_4, two hours after its administration. Only at this time, the rise in M.R. by T_4 approached a significance level of 0.05. At no other time did any treatment given produce a significant rise in M.R. The M.R. was depressed 24 hours after injection. The depressing effects of T_3 and $T_3 + T_4$ at this time were statistically significant.

No prior information is available regarding the effects of administration of T_3, $T_3 + T_4$ or T_4 on M.R. McCartney and Shaffner, (1950) and Mellen (1958), however, measured M.R. in thyroprotein-fed birds. The stimulating effect lasted for as long as supplementation was maintained or for a short time during the first few hours after fasting. Mellen (1958) also reported that M.R. was lower than in controls after 12-14 hours of fasting.

The effect of T_3 or T_3 in combination with T_4 is less than of T_4 alone. This is expected in view of the faster metabolism of T_3 than T_4. A short time action of the thyroid hormones on M.R. in chickens may be related to short biological half lives of these hormones as noted in the turnover experiments. The lower PBI levels in the chicken may be another factor worthy of consideration. Furthermore, differences of thyroxine-binding protein between birds and mammals may also be responsible for variation in thyroxine effect on M.R. The specific thyroxine-binding alpha-globulins

are not present in birds. There is no increase of M.R. in Rainbow trout following thyroxine administration (Fromm and Reineke, 1956).

Comparison of the TSR Methods

Only the goiter prevention and the thyroxine substitution method have been previously employed for estimation of TSR in chickens. In the present study, the direct output and the thyroxine degradation methods were also used, and four methods were compared. The values of TSR obtained in different experiments within each method were sufficiently close to indicate good repeatability. The average estimated TSR by the goiter prevention, the T_4 substitution, the direct output and the T_4 degradation methods, respectively, measure as 2.28, 2.00, 1.10 and 2.03 µg of T_4/ 100 g/day. Thyroid secretion rates measured by the substitution of T_4 on tapazole-treated birds and T_3 on both normal and tapazole-treated birds and the results of T_3 degradation are excluded from this comparison for the reasons discussed separately under each method.

Mellen and Wentworth (1960) compared goiter prevention and radio-iodine assay for determination of TSR in chickens. Mean values obtained by the former method were 65 percent of those derived from the latter method. They reasoned that the lower values from the goiter prevention assay resulted because the method was applied at higher

temperature and that because T_4 is administered on a per bird basis, but the final estimation of TSR is made by converting data on body weight basis. Tanabe et al. (1965) obtained equivalent values of TSR by employing goiter prevention and radioiodine assay methods. The radioiodine assay method adapted by Tanabe et al. is somewhat different and the values obtained by them are relatively lower than those generally reported by other workers. Further, a careful examination of their reports reveals that their experimental birds were fed on rations containing approximately 0.5 µg iodine per gram feed, which is rather low in view of the minimum iodine requirement of about 1.0 µg/gm in growing chickens (Wilgus, Gassner, Patton and Harshfield, 1953).

The use of the goiter prevention assay is based on the assumption that the estimated TSR is the dose of T_4 required to suppress the output of TSH in goitrogen-treated animals to that of the control. The variability of the goiter prevention assay was questioned from time to time (Escobar del Ray et al., 1962; Jagiello and McKenzie, 1960; Van Middlesworth et al., 1959). A serious objection has been in the use of goitrogen like thiouracil which decreases deiodination of thyroxine. This extrathyroidal action of the thiouracil is thought to give faulty TSR values. In view of this, methimazole, which has the least extrathyroidal effects, was used as the goitrogen in the present study.

Some other workers have, however, used both thiouracil and methimazole in the goiter prevention method and got identical TSR values in rats and chickens (Wiberg et al., 1964; Tanabe et al., 1965).

In case of the substitution method, an assumption is made that the TSR is the quantity of exogenous T_4 necessary to suppress thyroidal iodide release, or in other words, the T_4 dose which completely blocks TSH, is the TSR in this method. The values obtained by the substitution method in goitrogen-treated animals are undoubtedly higher as compared to any other method. Both the T_4 substitution and the goiter prevention assay, in which thyroxine is injected once daily, can be questioned on the ground that daily administration of thyroxine neglects important consideration of its short half life. In rats the plasma thyroxine concentration declines by about 75 percent in 24 hours after injections (Gregerman, 1963).

For chickens, the position will be still worse because the $t_{\frac{1}{2}}$ of thyroxine is much shorter than in rats. It may also be noted in the present study (Table 8) that the use of the T_4 substitution method in both normal and goitrogen-treated birds on diet deficient in iodine gave similar TSR values as in the birds on diet adequate in iodine. However, significantly lower values were obtained in chickens with iodine deficiency by use of the direct output

and the T_4 degradation methods. Similar results were observed in rats in comparison between the direct output and the T_4 substitution methods (Reineke, 1964).

It, therefore, seems that the direct output and the T_4 degradation methods should give more accurate estimations of TSR. Presently, however, these methods do not yield absolute values.

In the calculations for the direct output method, an assumption was made that the entire daily iodine output from the thyroid of the chicken is in the form of T_4. A factor of 1.529 for T_4 equivalent of iodine was used to convert the rate of iodine released daily into TSR. In fact, iodine may be released from the thyroid as T_4, T_3 and iodide with possibly some M.I.T. and D.I.T. The exact proportion of T_3 in the chick thyroid is not too well known. However, the results from the present study and the data from the other workers show that T_4 and T_3 are almost equally potent in chickens. The released iodotyrosines may be negligible. It is also believed by many workers that not more than 10 percent of iodine is released as iodide. In view of this, no other unknown factor, including T_3 was taken into account and the factor 1.529 was used. Presently, TSR values obtained by the direct output method are about half as large as observed in the other methods. Nevertheless, any error in this method that would arise if a portion of the iodine

released were in non-hormonal form (Iodide, M.I.T., D.I.T.) would result in overestimation rather than underestimation of the TSR. As pointed out earlier, there is too little difference between the potencies of T_3 and T_4 in chickens to account for a significant error in the calculations. Also, consideration of the known factors in this method indicate rather strongly that as presently applied, the direct output method most nearly represents the true TSR of the chicken.

The T_4 degradation method is delicate and there are, several factors involved which influence the TSR values.

I. The thyroxine distribution space may generally be overestimated as follows:

(1) I^{131} released from the deiodination of the labelled hormone is partly taken up by the thyroid and is partly excreted.

(2) Impurities in the commercially available I^{131}-T_4 and the possibility of the non-thyroxine component being more rapidly eliminated may affect the TDS.

(3) Increased radioactivity in the plasma samples may result due to efflux of unchanged hormone stored in the liver.

Numbers 2 and 3 were noted in the present study. Also, the thyroidal uptake of released I^{131} was checked for comparison by the administration of tapazole in a group of chickens. In this experiment, the estimated TSR approached

the values obtained by direct output (1.59 μg vs 1.10 by the direct output method).

II. The ratio of T_4 : T_3 as circulating thyroid hormones, the intensity of the plasma protein binding of the two hormones and the exact contribution of T_3 in the PBI are yet not fully understood in chickens.

The extent to which these unknown factors have influenced the estimated TSR in the present study is difficult to determine. It is, however, thought that when all the known and unknown factors have been properly accounted for, the values obtained by the T_4 degradation method should be close to those in the direct output method.

SUMMARY AND CONCLUSIONS

The thyroid secretion rate (TSR) in chickens of different ages has been determined by four methods: (1) goiter prevention, (2) thyroid hormone substitution, (3) direct output and (4) thyroxine (T_4) degradation. The results of these methods were compared. The values of TSR obtained in different experiments within each method were sufficiently close to indicate good repeatability. The representative estimated TSR by 1, 2, 3 and 4 methods respectively measure 2.28, 2.00, 1.10 and 2.03 μg/100 g/day. TSR of 7-week goitrogen-treated chicks, 56-week normal chicks, bobwhite quail and coturnix as estimated by the T_4 degradation method, respectively, averaged 1.59, 1.02, 2.49, and 2.78 μg/100 g/day. TSR estimated by the thyroxine (T_4) and triiodothyronine (T_3) substitution methods on goitrogen-treated chickens was much higher. There was no significant difference in TSR measured by the direct output method in growing chicks in the range of 1 to 9 weeks of age.

An increased plasma radioactivity was noticed in the 12-hour samples taken in the degradation experiments. It is presumed that such excessive radioactivity results due to discharge of unchanged hormone from the liver under a stress at that stage.

Effects of dietary iodine content on various parameters of TSR as determined by different methods and upon body and thyroid weights and thyroid iodine were determined. With diet adequate in iodine, TSR by the direct output method and the degradation method, thyroxine distribution space (TDS), extrathyroidal thyroxine (ETT), thyroidal iodine content and growth rate of chicks were significantly higher, but K_4, U uptake and thyroid weight were significantly lower. There was no difference of $t_{\frac{1}{2}}$, PBI and the TSR by the substitution method between the chickens on the diets containing adequate and inadequate iodine levels.

T_3 and T_4 were found to be almost equally potent by the method of substituting hormones to maximally block I^{131} release.

The representative biological half lives of T_4 in blood of chickens, bobwhite quail and coturnix were 3.23, 4.60 and 5.55 hours, respectively. The half life of T_3 in each kind of bird was not significantly greater.

The representative thyroxine distribution space in ml/100 g b.w. of chickens, bobwhite quail and coturnix, respectively, measured 29.39, 28.08 and 55.29. TDS/unit body weight of 56-week old chickens is significantly lower than in 7-week chickens. T_3 distribution spaces in all birds are higher than of T_4.

The representative protein-bound iodine of chickens, bobwhite quail and coturnix were measured as 1.12, 1.76 and

1.26 µg percent, respectively. PBI of bobwhite quail is higher than of chickens and coturnix.

Tapazole (a goitrogen) retarded growth rate, feather and comb growth of chickens. Thyroxine in the doses of 2-3 µg/100 g/day counteracted completely the growth inhibiting effects of tapazole. Thyroxine in small doses (0.5 to 4.0 µg/100 g/day) improved growth of normal chickens. But, 6 µg of the hormone depressed growth of normal and 4 µg depressed growth of goitrogen-treated birds.

T_4, T_3 or a combination of T_4 + T_3 produced a small and transitory rise in M.R. of chickens. Only at 2 hours after T_4 administration did the rise in M.R. approach the 0.05 significance level. The depressing effect of T_3 and T_3 + T_4 at 24 hours after their injection was found to be significant.

The following indices of thyroid function were found to be significantly related:

Log K_4 and log age. $r = -.8351$, $P < .005$.

Thyroidal iodine and age. $r = .8869$, $P < .005$.

Log K_4 and log thyroidal iodine.
$r = -.8285$, $P < .005$.

Log K'_4 and U uptake. $r = -.6719$, $P < .005$.

Log thyroidal iodine and log body weight in growing chicks. $r = .9784$, $P < .005$.

Thyroidal iodine and thyroid weight in adult chickens and quail. $r = .9416$, $P < .005$.

T_3 distribution space and body weight of chickens (Spearman rank correlation coefficient)
$rs = .9023$, $P < .02$.

The data in the present studies apart from other observations support the conclusion that the thyroid function in chickens differs in general from that of mammals. Chickens have lower PBI values, shorter half-lives of T_3 and T_4 in the plasma, almost equal physiological potency of T_3 and T_4 and show an insignificant rise of M.R. following administration of the thyroid hormones.

The merits and demerits of the four TSR methods used in this project have been discussed. It is thought that the direct output and the degradation methods should yield values closer to TSR, provided that all their known and unknown factors are properly accounted for in the calculations. However, consideration of the known factors involved in these methods indicates rather strongly that as presently applied, the direct output method most nearly represents the true TSR of the chicken.

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APPENDIX A

DATA ON TSR OF INDIVIDUAL CHICKS BY DIRECT OUT-PUT METHOD

Chick No.	Weight gms.	U Percent Maximum Uptake	K'_4	K_4	Total Thyroidal Iodine µg	TSR/ 100 gm b.w. /day
1-week-old						
401	88	.014	.1627	.1650	1.84	.5268
402	90	.034	.1555	.1610	2.71	.7387
403	90	.024	.2264	.2320	2.48	.9761
404	85	.041	.2379	.2480	3.65	1.6274
405	100			.5214	3.10	2.4705
406	97			.2469	5.46	2.1260
407	103	.084	.2125	.2319	1.87	0.6446
408	78	.036	.3604	.3738	1.26	0.9246
409	87	.021	.1975	.2017	3.19	1.1590
410	95	.046	.3347	.4272	1.18	0.8112
3-week-old						
189	184	.106	.0819	.09166	13.63	1.040
190	240	.110	.0861	.09670	10.28	0.630
191	230	.150	.0703	.08271	12.82	0.700
192	242	.069	.1521	.16338	17.43	1.800
195	190	.028	.2063	.21221	3.41	0.580
196	218	.055	.1344	.14220	6.42	0.640
197	140	.072	.0930	.10020	6.21	0.670
198	210	.062	.2601	.27734	4.57	0.920
199	170	.076	.0810	.08769	8.17	0.640
200	182	.074	.1026	.11075	6.61	0.610
11		.055	.1307	.1383	15.68	
12		.056	.1131	.1198	11.55	
13		.049	.1131	.1189	7.39	
14		.062	.0957	.1020	24.81	

Chick No.	Weight gms.	U Percent Maximum Uptake	K'_4	K_4	Total Thyroidal Iodine µg	TSR/ 100 gm b.w. /day
15		.093	.1220	.1345	11.72	
16		.062	.1393	.1485	13.37	
17		.048	.1307	.1372	14.96	
18		.052	.1220	.1287	16.32	
19		.088	.1131	.1240	15.66	
20		.063	.1220	.1302	8.36	
4.5-week-old						
184	404	.082	.0810	.0883	31.25	1.043
122	462	.093	.0718	.0792	44.54	1.166
199	450	.051	.1270	.1338	51.90	2.359
187	452	.024	.1344	.1376	26.55	1.235
185	395	.037	.1707	.1773	21.67	1.487
193	360	.069	.0955	.1026	19.35	0.843
182	420	.098	.0683	.0756	36.40	1.002
110	450	.082	.0810	.0883	52.57	1.576
181	430	.054	.1204	.1273	22.20	1.005
108	363	.070	.0951	.10223		
4.5-week-old (Diet deficient in iodine)						
27	568	.085	.2420	.2646	6.81	.4853
28	515	.090	.2473	.2717	7.16	.5774
29	550	.096	.1086	.1201	10.84	.3619
30	414	.090	.1324	.1454	4.63	.2481
31	396	.062	.0979	.1043	9.37	.3772
32	371	.110	.1918	.2156	6.16	.5473
33	523	.082	.1263	.1375	10.12	.4069
34	294	.072	.1537	.1656	6.16	.5302
35	427	.098	.2181	.2417	9.38	.8117
36	455	.140	.1157	.1346	6.87	.3108

Chick No.	Weight gms.	U Percent Maximum Uptake	K'_4	K_4	Total Thyroidal Iodine µg	TSR/ 100 gm b.w. /day
9-week-old						
101	1475	.210	.0310	.03921	270.00	1.09
102	1475	.110	.0325	.0365	291.25	1.10
103	1475	.160	.0703	.0837	131.25	1.13
104	1425	.091	.0297	.0326	108.12	0.38
105	1440	.125	.0273	.0312	216.87	0.72
106	1720	.110	.0208	.0233	228.12	0.47
107	1350	.097	.0703	.0778	144.68	1.27
108	1350	.190	.0267	.0330	231.87	0.86
109	1450	.280	.0741	.1029	248.75	2.69
110	1400	.145	.0284	.0332	294.68	1.06

APPENDIX B

THYROXINE TURNOVER IN INDIVIDUAL BIRDS

Bird No.	Weight gms	t ½ Hours	TDS ml/100 gm b.w.	PBI µg %	ETT µgI* (TDS/100 gm b.w. x PBI µg/ml.)	Thyroxine degradation µg/100 gm b.w./day
Chickens: 5-6-week-old (Diet deficient in iodine)						
24	580	4.1	8.61	1.0538	.09073	.5178
25	430	3.0	8.98	.7762	.06970	.5273
33	485	5.1	10.89	1.6315	.17767	.8271
40	360	3.0	12.39	1.3003	.16111	1.2190
42	260	4.1	15.70	.9475	.14876	.8488
37	355	3.0	5.75	1.0836	.06231	.4715
30	375	4.0	12.63	1.0584	.13367	.7798
38	395	4.6	11.34	1.0886	.12345	.6334
Chickens: 6-7-week-old						
6	605	3.0	34.52	.6854	.23660	1.79
20	530	3.5	34.67	.9937	.34451	2.27
45	705	3.0	23.28	.8717	.20293	1.54
46	660	3.0	24.86	1.1245	.27955	2.11
48	675	4.5	45.33	1.0800	.48956	2.56
53	732	3.5	36.92	.8820	.32563	2.15
58	710			1.0314		
59	765	4.25	50.05	.9759	.48843	2.69
60	660	3.0	25.78	.6860	.17685	1.34
95	840	3.25	29.56	.8034	.23748	1.80
339	744	3.3	26.84	1.1440	.30704	2.13
330	564	3.7	28.58	.8360	.23893	1.49
338	572	3.0	36.51	1.0120	.36949	2.79
344	692	3.4	28.86	.9900	.28576	1.93
337	568	2.9	32.35	.8250	.26695	2.08
328	732	3.1	23.24	1.0450	.24287	1.78

Bird No.	Weight gms	t ½ Hours	TDS ml/100 gm b.w.	PBI µg %	ETT µgI* (TDS/100 gm b.w. x PBI µg/ml.)	Thyroxine degradation µg/100 gm b.w./day
Chickens: 7-week-old, goitrogen treated						
332	560	2.4	17.64	1.0890	.19215	1.76
333	590	3.3	25.95	1.0450	.27121	1.88
346	732	2.5	19.61	1.0560	.20709	1.84
345	610	3.1	18.83	.8580	.16156	1.15
336	752	2.8	20.36	.8690	.17697	1.42
348	552	3.0	20.81	.9680	.20140	1.52
Chickens: 56-week-old						
7095	2120	3.6	20.64	.8140	.16801	1.07
7096	1935	4.7	10.79	.8690	.09376	.47
7097	2237	4.2	9.78	1.2650	.12369	.69
7093	2285	3.7	10.58	1.110	.11751	.73
7080	2110	3.7	27.56	1.2430	.34257	2.14
7092	2267	3.3	7.79	1.8920	.14744	1.03
Bob white quail: 56 - 68-week-old						
1535	166.6	3.8	17.79			
6053	177.3	3.8	19.19	1.2210	.23429	1.41
3771	183.7	4.7	40.66	2.5810	1.04950	5.31
295	262	6.0	21.92	1.3690	.30005	1.21
281	193	4.4	25.06	1.9140	.47966	2.58
3600	188	5.6	37.07			
290	173	4.7	21.25	2.8610	.60798	3.28
6612	211.5	3.8	28.03			
296	211	4.4	31.10	1.2210	.37971	2.04
1219	180.4	3.5	30.87	1.440	.44451	2.95
6477	178.5	5.3	25.71	.9680	.24890	1.12
12174	176	3.5	18.65			
12165	177	5.41	7.31			

Bird No.	Weight gms	$t_{\frac{1}{2}}$ Hours	TDS ml/100 gm b.w.	PBI µg %	ETT µgI* (TDS/100 gm b.w. x PBI µg/ml.)	Thyroxine degradation µg/100 gm b.w./day
12033	190	3.75	12.72			
12018	162	5.0	29.85			
10581	203	5.33	18.10			
Japanese quail: 10-week-old						
293	114	4.5	67.17	2.3410		
297	97.5	3.9	66.37	.8000	.53096	3.17
284	97.7	3.8	72.35	.9460	.68445	4.18
291	118.8	4.0	57.29	1.2000	.68748	4.01
299	109.5	8.0	52.45	1.1650	.61100	1.86
280	107.5	4.1	55.14	.9860	.53375	3.04
287	109.2	4.4	43.15	1.0560	.45572	2.43
276	115.5	8.0	49.73	1.4410	.71656	2.18
288	95.5	8.2	45.82	1.0120	.46371	1.38
300	92.7			1.0000		

*Multiply with T_4 iodine equivalent (1.529) to convert into µg T_4.

APPENDIX C

TRIIODOTHYRONINE TURNOVER IN INDIVIDUAL SUBJECTS

Bird No.	Weight gms	t ½ Hours	TDS ml/100 gm b.w.	ETT µgI* (TDS/100 gm b.w. x PBI µg/ml)	PBI µg %
Chickens: 7-week-old					
331	554	4.0	60.16	.71470	1.1880
421	660	3.7	68.87	1.06059	1.5400
425	612	3.7	65.36	.84118	1.2870
347	682	4.0	50.56	.80643	1.5950
342	582	4.0	58.04	.72782	1.2540
329	680	3.2	59.78	.72333	1.2100
Chickens: 7-week-old goitrogen treated					
326	752	3.8	53.19	.70795	1.3310
343	664	3.6	65.47	.87140	1.3310
327	824	4.2	69.74	1.31808	1.8900
335	912	4.3	57.71	.66655	1.1550
341	526	3.8	52.22	.55718	1.0670
340	406	3.5	84.93	1.07436	1.2650
Chickens: 56-week-old					
7090	2315	10.4	79.99	1.07346	1.3420
7100	2415	9.8	53.08	.56636	1.0670
7085	2057	14.4	67.52	.95811	1.4190
7098	2117	8.3	51.34	.72286	1.4080
7088	1775	6.4	65.51		4.0150
7091	2295	6.8	50.66	.85818	1.6940
Bobwhite quail: 56 - 68-week-old					
3857	185.3	5.5	59.96	.79807	1.3310
4753	175.6	10.0	79.08	.88569	1.1200
3020	194.0	3.8	55.42	.48160	.8690

Bird No.	Weight gms	t ½ Hours	TDS ml/100 gm b.w.	ETT µgI* (TDS/100 gm b.w. x PBI µg/ml)	PBI µg %
4950	169.5	11.4	59.59	.83247	1.3970
12088	164.0	9.5	64.18	2.7148	4.2300
5135	189.6	6.7	67.61	.77346	1.1440
13555	186.8	8.8	68.63	1.72878	2.5190
3409	200.0	9.3	64.93	1.08562	1.6720
3139	182.1	3.5	61.01	.87244	1.4300
3683	187.6	6.8	74.03	1.7340	2.3430
Japanese quail:		10-week-old			
283	94.0	4.1	99.41	2.36198	2.3760
286	94.6	4.5	62.17	.77961	1.2540
294	107.2	4.2	79.04	.92161	1.1660
279	89.1	4.1	102.02	1.52622	1.4960
277	94.1	4.2	96.60	1.03072	1.0670
278	100.6	4.4	124.25	1.61276	1.2980
450	85.2	4.5	73.35	.95208	1.2980
285	113.5	4.4	102.45	1.19456	1.1660
139	82.4	4.3	102.84	1.76576	1.7176
N.B.	92.0				1.2980

*Multiply with T_3 iodine equivalent (1.71) to convert into µg T_3.

APPENDIX D

ADDITIONAL DATA ON THYROID IODINE AND PBI OF CHICKENS AND QUAIL

Bird No.	Weight gms.	Thyroid Weight mg.	Total Thyroidal Iodine µg	PBI µg %
7-week-old chickens (chick starter diet)				
21	695	34.0	74.81	
22	730	47.0	59.38	
23	750	37.0	49.81	
24	640	72.0	163.69	
25	765	62.0	105.31	
26	775	47.0	80.88	
27	720	61.5	143.19	
28	685	65.0	103.13	
29	660	39.5	35.38	
30	795	52.5	89.44	
7-week-old chickens (Diet SR-2 added I, 1.3 µg/gm feed)				
1	718	30.0		1.0670
2	760	52.0	33.69	1.2760
3	620	36.0	33.00	1.0010
4	770	37.5	59.12	1.3420
5	780	57.0	92.12	1.2870
6	770	54.0	49.02	1.3420
7	695	57.0	113.44	1.1550
8	575	47.0	81.40	1.4300
9	700	40.5	42.35	1.2100
10	642	46.0	31.56	1.2100
7-week-old chickens (Diet SR-2 with no I added)				
11	665	48.5	3.91	.9900
12	600	50.0	2.61	.9350

Bird No.	Body Weight gms.	Thyroid Weight mg	Total Thyroidal Iodine µg	PBI µg %
13	520	55.0	0.73	.9130
14	650	114.0	1.03	.9900
15	582	64.0	1.45	.6930
16	680	72.0	5.69	.9570
17	645	95.0	5.98	1.1220
18	723	47.0	1.76	.8690
19	623	75.0	3.13	.9130
20	685	100.0	4.37	.9240
11 week-old-chickens (Adequate iodine diet)				
1	1415	97.0	284.46	
2	1400	117.0	387.75	
3	1100	81.5	158.73	
4	1827	194.5	338.25	
5	1352	115.0	383.46	
6	1434	150.0	344.52	
7	1203	78.0	283.80	
8	1270	76.0	181.50	
9	1365	100.0	191.73	
10	1404	73.0	168.96	
13 week-old-chickens (Adequate iodine diet)				
1	1450	111.5	342.37	
2	2035	99.5	544.50	
3	1372	156.0	484.60	
4	1668	131.5	455.07	
5	1332	130.0	387.25	
6	1635	93.0	281.32	
7	1851	191.0	427.02	
8	1531	180.2	492.52	

Bird No.	Body Weight gms	Thyroid Weight mg.	Total Thyroidal Iodine µg
9	1554	92.0	220.27
10	1556	102.3	375.87
14-month-old chickens (Adequate iodine diet)			
7093	2285	165.0	613.80
7096	1935	179.0	575.47
7095	2120	122.0	479.16
7097	2237	183.0	511.17
7088	1775	83.0	244.20
7091	2295	179.0	560.34
7090	2315	166.0	490.77
7098	2117	105.0	362.12
7100	2415	167.0	406.34
7080	2110	127.5	535.70
14-17-month-old Bobwhite quail (Adequate iodine diet)			
3857	185.3	9.5	17.55
4753	175.6	12.0	19.95
3020	194.0	9.0	19.12
4950	169.5	12.0	33.75
12088	164.0	9.0	21.07
13555	186.8	8.5	332.40
5135	189.6	44.5	7.65
3409	200.0	26.5	118.65
3139	182.1	14.5	21.02
3681	187.6	29.0	115.13
281	193.0	13.5	51.35
1219	180.4	12.0	27.97
296	211.0	11.5	41.40
295	262.0	13.0	46.87
3771	183.7	9.5	18.82

Bird No.	Body Weight gms.	Thyroid Weight mg.	Total Thyroidal Iodine µg
6612	211.5	49.0	237.87
290	173.0	16.0	61.17
6477	198.5	18.0	97.50
3600	188.0	18.0	88.87
1535	166.6	14.5	62.50
6053	177.3	12.5	57.10
10-week-old Japanese quail (Adequate iodine diet)			
283	94.0	7.0	23.20
286	94.6	8.0	21.87
294	107.2	8.0	26.25
279	89.1	5.5	16.36
277	94.1	4.5	7.07
278	100.6	5.0	7.49
450	85.2	4.5	13.69
285	113.5	5.0	16.25
139	82.4	8.5	33.75
N.B.	93.0	11.5	58.20
276	115.5	5.5	18.24
284	97.7	6.5	15.49
287	109.2	4.0	28.62
300	92.7	6.5	13.01
280	107.5	7.5	37.42
291	118.8	3.0	6.80
293	114.0	6.0	16.95
297	97.5	4.0	1.87
288	95.5	4.0	2.67
299	109.5	3.5	3.35

APPENDIX E

THYROID IODINE ANALYSIS

Method of Barker and Humphrey (1950) for analysis of protein-bound iodine has been modified for determining total thyroidal iodine (Reineke, unpublished).

Iodine Determination

Place 1 cc of 4 N Na_2CO_3 into a pyrex test tube containing the thyroid. Dry overnight at 90-95°C. Dried residue is then incinerated in muffle furnace for 2½ hours at 600-625°C.

Dissolving iodine from ash

Add 2 cc 2N HCl
2 cc 7 N H_2SO_4 and
*21 cc glass distilled water.
Mix and stir until no more effervescence appears.
Centrifuge for 20 minutes.

Colorimetry

Treat each sample and the reagent blank in duplicate. Take *1 cc of the digest into colorimeter tube. Add *4 cc glass distilled water and 0.5 cc arsenious acid reagent (Hycel). Place in water bath at 27°C. Allow tube a few minutes to reach that temperature. Add 0.5 cc dilute** ceric ammonium sulphate reagent (Hycel) at 30-second intervals to each tube. Let stand in bath exactly 15 minutes after addition of ceric ammonium sulphate. Then add 0.5 cc of 1% brucine solution at 30-second intervals. Read samples in Coleman spectrophotometer at a wave length of 480 milli-microns. Subtract regent blank value and read final value in µg of iodine from the standard curve*** prepared earlier at the same temperature and duration of reaction.

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*May vary according to the expected iodine content in the aliquot.

**2 cc Hycel reagent diluted to 5 cc with glass distilled water.

***Curve for high iodine value at 27°C for 15 minutes ranges from 0 to 0.2 µg of iodine.

APPENDIX F

PBI ANALYSIS

The method is similar to the thyroid iodine analysis.

Plasma Protein Precipitation

1 cc plasma + 7 cc glass distilled water + 1 cc of 10% $ZnSO_4$ + 1 cc 0.5 N NaOH. Add NaOH slowly with constant stirring to aid in the formation of the precipitate. Wait for 5 minutes. Centrifuge, discard supernatent liquid and wash protein thrice with 10 cc glass distilled water. Centrifuge and discard supernatent each time.

In order to eliminate any free iodine that may affect PBI analysis, dual precipitation was done as follows:

1 cc plasma + 10 cc glass distilled water + 4 cc 11.2% trichloroacetic acid. Wash precipitate once with 3% TCA, dissolve in 7 cc glass distilled water. Add 1 cc $ZnSO_4$ solution and 1 cc NaOH to reprecipitate proteins. Centrifuge and wash the precipitate.

Drying and Ashing

Dissolve precipitate in 1 cc 4 N Na_2CO_3. Dry overnight at 90°C. Incinerate at 600-625°C for 2½ hours.

Dissolving iodide from ash

Add 2 cc 2 N HCl
2 cc 7 N H_2SO_4 and
*7 cc glass distilled water. Stir and centrifuge.

Colorimetry

Take *5 cc aliquot in duplicate. Add 0.5 cc arsenious acid reagent (Hycel). Place in water bath at 50°C. Allow tubes a few minutes to reach that temperature. Add 0.5 cc dilute** ceric ammonium sulphate (Hycel) at 30-second intervals to each tube. Let stand in bath exactly 20 minutes after addition of ceric ammonium sulphate. Stop reaction with 0.5 cc 1% brucine solution. Read in colorimeter. Subtract blank value and read final value in µg of iodine from the standard curve*** prepared at the same temperature and duration of reaction.

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*May vary according to the expected iodine content in the aliquot.

**2 cc Hycel reagent diluted to 5 cc with glass distilled water.

***Curve for low iodine value at 50°C for 20 minutes ranges from 0 to 0.05 µg iodine.

APPENDIX G

MICHIGAN STATE UNIVERSITY

63-S Chick Starter
KRUMBLES

Fine Ground Yellow Corn	1060
Ground Oats (38-40#/bushel)	100
Wheat Middlings	100
17% Dehydrated Alfalfa Meal	80
50% Meat/Bone Scraps	50
45% Protein Soybean Meal	500
55% Fish Meal Vitaproil	40
Dried Whey	40
Ground Limestone	10
Dicalcium Phosphate (24% Calcium 18.5% Phosphate)	10
Salt, Iodized	6
Vitamin Trace Mineral Premix	5-10
NFZ added	-
	2001 +

Manufactured by
KING MILLING COMPANY
LOWELL, MICHIGAN

APPENDIX H

RAT FEED MIXTURE SR-1

Add 0.5 lbs. of special mineral salt premix SR-1 per each 100 lbs. of basic feed mix and mix thoroughly.

A. Basic Feed Mix:

Ingredient	Amount/100 lbs. of Mix
Shelled Yellow Corn ground through 1/8" screen	68.9 lbs.
Soya-bean oil meal (50% protein)	28.0 lbs.
Dicalcium phosphate	1.8 lbs.
Lime stone	0.6 lbs.
Dawes & Forbes Vitamin B supplement	0.1 lbs.
Dawes & Forbes Vitamin B_{12} supplement	0.2 lbs.
Standard Brands 9F yeast 9000 I.V. (Vit. D_2/g)	5.0 gm
Pfizers Vitamin A supplement (10,000 I.V. Vit. A/g)	15.0 gm

B. Mineral Salt Premix SR-1:

Element	% Element	Compound	gm/100 lbs.
*Iodine	0.010	KI-10% Ca Stearate (Pfizer)	0.653
Zinc	0.800	$ZnSO_4.7H_2O$ (Baker)	160.574
Manganese	0.542	$MnSO_4.H_2O$ (Baker)	73.12
Iron	0.270	$FeSO_4.7H_2O$ (Baker)	60.782
Copper	0.054	$CuSO_4$. (anhydrous Baker)	6.169
Sodium Chloride (plain)		(Morton's)	4234.72

*This feed mixture minus iodine is termed SR-2.

APPENDIX I

DIET FED TO 56-WEEK OLD CHICKENS

Corn - free choice plus

Ground Yellow Corn	1320 lbs
Soybean Meal, dehulled, 50% protein	310
Alfalfa meal, dehyd. 17% protein	60
Meat & Bone Scraps 50% protein	50
Fish Meal 55% protein	60
Dried Whey	40
Ground Limestone	100
Dicalcium phosphate	20
Salt, iodized	6
Vitamin trace mineral premix (contains iodine)	10
Choline chloride 25%	2
Zinc oxide (80% zinc)	0.25
Animal Fat	25
	2003.25 lbs

APPENDIX J

DIET FED TO QUAIL
Protein 25%

Ground Yellow Corn	412.5
Soymeal dehulled 50% prot.	370.0
17% Alfalfa Meal	50.0
Dried Whey	25.0
Meat/Bone Scraps	25.0
Fishmeal (Menhaden) 60%	25.0
Ground Limestone(CaC03)	50.0
Dicalcium phosphate	15.0
Salt Iodized	5.0
Vit. Premix 1	
Nopcosol M-4 (contains iodine)	2.5
Fat	20.0
	1000 lbs.

APPENDIX K

EFFECT OF A SINGLE DOSE OF (4.0 µg 100 gm b.w.) THYROID HORMONES ON M.R. IN CHICKENS. O_2 Consumption ml/hour/kg. b.w.

Treatment	Chick Number	Hours after Injection		
		1	2	7
Control	326	1086	1237	1474
	327	1182	1552	861*
	328	1271	1468	1446
	329	1313		1495
	330	1547	1516	1595
	331	1795	1483	1380
	332	1553	2180	1540
	333	1163	2270	1619
T_4	334	1203	2383	1627
	335	1150	1659	1138
	336	1505	1944	1402
	337	1370	1917	1325
	338	1584	2220	1538
	339	1220	2160	1512
	340		2042	1770
	341	1442	2068	1399
T_3	342	1611	1697	1679
	343	1754	2055	1649
	344	1386	2174	1376
	345	1209		1503
	346	1487	1854	
	347	1479	1653	1623
	348	1573	2010	898*
	349	1970	1878	1921

*Eliminated from statistical analysis by Chauvent criterion. Documenta Geigy. Scientific tables, 5th Edition, p. 47.

(Body weight of chickens 250-350 gm.)

APPENDIX L

EFFECT OF DAILY ADMINISTRATION OF (6.0 µg/100 gm b.w.)

THYROID HORMONES ON M.R. IN CHICKENS.

O_2 Consumption ml/hour/kg. b.w.

Treatment	Chick Number	Days of Administration			
		15	14	7	11
		Hours after injection			
		2	3	24	24
Control	151	1746	2006	2256	1828
	152	1812	1922	1963	1692
	153	1567	1697	1469	1905
	154	1971		1940	
	155	1620	1663	1811	1810
	156	1666	1528	1569	2117
T_4	157	1755	1302	1614	2074
	158	1826	1902	1921	2169
	159	2236	1844	1598	2099
	160		1537	2032	
	161	1495	1720	1754	1979
	162	1779	2149	2066	1645
T_3	163	1887	1709	1370	1681
	164	1859	1456		
	165	1894	2026	1518	1661
	166	1577		1163*	1568
	167	1646	1723	1448	1571
	168	1688	1824	1811	1624
$T_3 + T_4$	170	1753	1901	1120	2066
	171	1347	1944	1029	1636
	172	1909	1622	1695	1489
	173		2400*	1543	1760
	174	1903	1949	1608	1899
	175	1512	1825	1599	1110

*Eliminated from statistical analysis by Chauvent criterion. Documenta Geigy. Scientific tables, 5th Edition, p. 47.

(Body weight of chickens 120-225 gm.)