Comparative Thyroid Function in Developing and Adult Precocial Japanese Quail and Altricial Ring Doves.

by

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(ABSTRACT)

I compared Japanese quail *Coturnix japonica*, and Ring doves *Streptopelia risoria*, in the development of thyrotropin (TSH) influence on thyroid hormone (TH) content in the thyroid gland (TG) and the serum, and on TG-cAMP content. In embryos, pituitary gland (PG)-TSH content was measured by an avian bioassay system. Adult quail and doves were studied for comparability of thyroid gland function when receiving comparable dietary iodine. Also, thyroid function in adult doves was compared with different iodine intake.

In embryonic quail, there is considerable maturation of thyroid function prior to hatching. TG-TH content is low but detectable in day 8 embryos; TG-TH content increases 300X between day 8 and hatching (16.5 day incubation). Pituitary TSH was detectable on embryonic day 8, with higher levels found closer to hatching. The TG of 8 day embryos responds to TSH injection by increased TG-cAMP content but the serum TH response to TSH does not mature until day 9. Serum TH concentrations suggest that the TG is under the control of endogenous TSH from the pituitary during the latter part of incubation.

In doves, most of the development of thyroid function and the maturation of its pituitary control occur after hatching. Thus, thyroid functional development is much later in doves than in quail. TG-TH content is extremely low in embryos and nestlings up to 3 days after hatching, increases slowly in nestlings up to day 10, then increases sharply. Serum TH are very low in embryos and rise steadily in nestlings to plateau after day 8. Pituitary TSH content is undetectable in

embryos and in nestlings until day 4. The TG does not respond (based on serum TH concentrations) to TSH injection through the day of hatching (day 16; mean incubation period of 16.5 days), but an increase in serum TH occurs in day 2 nestlings in response to TSH injection. The magnitude of this response continues to increase during the first week after hatching.

In adult birds, thyroid function was studied in Japanese quail and Ring doves, when both were fed the same dietary iodine (I; 930 μg I/kg). We also compared thyroid function in groups of doves receiving low I (< 100 μg I/kg) or moderate I (930 μg I/kg). We measured thyroid gland (TG) weight, TG stable I (^{127}I) I content, TG ^{125}I uptake, ^{125}I labelling of thyroid hormones, and serum ^{125}I thyroxine (T4) half-life. Triiodothyronine (T3) and thyroxine (T4) concentrations in TGs and serum also were determined.

Our results indicate that doves and quail receiving the same dietary I show similar serum T3 concentrations and TG functional state, but that there are some differences between the species in the way which this equivalent functional state is achieved. Doves fed low dietary I (< 100 μg I/kg) when compared to doves with moderate I intakes (930 μg I/kg) showed similar serum T3 concentrations despite reduced serum T4 concentrations and TG-hormone stores. This study demonstrates that quail and doves show similar TG function and a similar regulation of serum T3, the presumed metabolically active hormone, when dietary I availability is the same. Also, doves with low dietary I show decreases in some measures of TG function compared to doves with moderate I, but still maintain a level of serum T3 comparable to that with adequate I intake. This set point regulation of T3 therefore appears to be independent of serum T4 or TG hormone stores.

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CHAPTER ONE: INTRODUCTION

This study of thyroid function in Japanese quail and Ring doves is divided into two sections. The first is a comparison of the development of thyroid function and its control in the two species by the pituitary gland in the embryonic and nestling stages of both species. The differences in the timing of the maturation are related to the different developmental modes in the two species. In the second part of the study, thyroid function in adult quail and doves were compared when both species received the same dietary iodine availability. In addition, I examined the influence of dietary iodine on thyroidal function in adult doves receiving low or moderate iodine intakes. The relationship of these two manuscripts to the overall research goals of the laboratory are discussed in the significance section.

Literature Survey

Altricial vs. Precocial Modes of Development

The attainment of thermoregulatory control in endothermic animals occurs by two different patterns. Precocial species, including the galliforms, are born/hatched in a relatively advanced state. They show endothermic responses to cooling early and are active, have good locomotor skills and are relatively independent of parental care (Nice, 1962). Altricial species, e.g. columbiforms are born/hatched in a much less advanced state. They show little endothermic response to cooling and are dependent on parental brooding. In addition, they lack physical coordination and are dependent on parental feeding and care (McNabb, 1987). (Among avian species, galliform birds such as chickens and quail are precocial; passeriforms such as sparrows and columbiforms such as doves are altricial). Physiological studies of thyroid function in birds have focused on the precocial galliforms (especially chickens and quail), while their have been a limited number of studies in altricial doves.

Precocial Thyroid Function

Much of the work on thyroid functional development in avian species has been on Japanese quail and chickens. In both species, the thyroid gland is organized into follicles toward the end of the first half of the incubation period (quail: day 8-10 of 16.5 day incubation, McNabb and McNabb, 1977; chicken: day 8-11 of 20.5 day incubation, Thommes and Tonetta, 1979). This is followed rapidly by the increased production and release of TG hormones (quail: McNabb et al., 1984a; McNichols and McNabb, MS in prep; chickens: Thommes and Tonetta, 1979). In quail, TG function between days 8 and 10 is low but there are gradual increases in TG-T3 and TG-T4 at this time. This is followed by a greater than 20X increase in TG-T4, that occurs between days 11 and 15 of incubation (McNabb, 1987). Thyroidal T4 content increases more rapidly than TG-T3 content with TG-T3/T4 ratios decreasing from day 8 to hatching (McNichols and McNabb, MS in prep). During the last third of incubation, embryonic quail and chickens show increases in TG hormone production and storage that exceeds the initial rate of hormone release leading to increased TG hormone stores which support the perinatal peak of serum hormone concentration.

The serum TH concentrations in quail are low from days 8-10 of incubation, then there is a linear increase in TH concentrations until day 15 (McNichols and McNabb, MS in prep). This is followed by a dramatic increase in the serum T4 concentration with a peak occurring in the perinatal period (McNabb et al., 1981). During the early embryonic period (days 8-11) the serum concentration of T3 remains consistently low . A rapid rise in serum T3 concentrations begins on day 15; there is a peak during the perinatal period, and relatively high serum T3 concentrations continue in the posthatching period (McNabb et al., 1981).

Chicken embryos show a serum T4 pattern comparable to that of quail, with increasing concentrations from days 9.5 to 19.5 of incubation and a perinatal peak (Thommes et al., 1977). The pattern of serum T3 is one of gradually increasing concentrations from day 11.5 to day 15.5,

followed by marked increases through the perinatal period (Thommes and Hylka, 1977; King et al., 1977).

Pituitary Control of Thyroid Function

Thyroid function appears to be independent of pituitary control in both chickens and quail until the second half of incubation (quail: McNabb et al., 1984a; chickens: Thommes and Tonetta, 1979). In quail, the thyroid gland becomes sensitive to exogenous TSH by embryonic day 9 (McNichols and McNabb, MS in prep). This is consistent with the finding in day 9 quail of evidence of maturation of the feedback response by the P-T axis to decreases of serum TH with goitrogen treatment (McNabb et al., 1984b).

In chickens, thyrotropes first are demonstrable on day 6.5 of incubation with significant production of TSH occurring on day 11.5 (Thommes et al., 1983). The embryonic chick thyroid was shown to be sensitive to TSH injection as early as day 6 as indicated by an increase in colloid formation (Tixier-Vidal, 1956). In another study, adenohypophyses of embryonic chickens of day 6.5 were found to respond to exogenous TRH by stimulating the TG with a resulting higher serum T4 concentration (Thommes et al., 1984). This indicated that the thyroid, as well as the pituitary gland, of the chick embryo is sensitive to its respective tropic hormones by day 6.5 of incubation. Thus, the finding of Thommes and Hylka (1978) of increases in circulating serum T4 concentration and increases in TG radioiodine uptake in day 10.5 and 11.5 embryos compared to earlier stages, are not reflecting the initial maturation of the P-T axis but are probably related to a completion of maturation of the HP-T axis and the commencement of hypothalamic release of TRH. This is supported by the finding of changes in the magnitude of response of chick embryo adenohypophyses to exogenous TRH, (as evidenced by increases in serum T4 concentration), during days 10.5 to 11.5 of incubation. Hypophysectomy and transplantation experiments and goitrogen feedback experiments also demonstrate that the P-T axis becomes functionally mature between days 10.5 to 11.5 in chickens. (Thommes and Tonetta, 1979; Thommes and Jameson,

1980). The completion of maturation of the HP-T axis during the embryonic period allows the dramatic increases in TG hormone stores that occur during the late embryonic period in precocial species. These stores support the peak in serum concentrations that occurs in precocial embryos during the perinatal period (quail: McNabb et al., 1981; chickens: Davison, 1976; Thommes and Hylka, 1977) and maintain the relatively high serum TH concentrations of chicks (McNabb et al., 1984a).

Altricial Thyroid Function

There has been little work on thyroid function in altricial species of birds. Thyroid follicles have been demonstrated in altricial doves at about the same time as in quail (embryonic day 8-10; McNabb and McNabb, 1977) but functional development of the thyroid occurs much later in the doves (McNabb et al., 1984a; McNichols and McNabb, MS in prep). The serum TH and TG contents during the embryonic period are extremely low with no evidence of a perinatal peak in serum TH, and with only slight increases in thyroid function in the last three days of incubation (McNichols and McNabb, MS in prep). The embryonic patterns of activity are consistent with the low levels of thyroid function in early nestlings with low gradually increasing serum concentrations and stabilization at adult levels at about days 6-8 (McNabb and Cheng, 1985; McNichols and McNabb, MS in prep). There is evidence that the increases in serum hormone concentrations of T3 and T4 precede the time of marked increases in TG stores of the hormones (day 10). Thus it appears that hormone release parallels the production of hormone, with little hormone storage until 10 days after hatching. That is followed by a period of TG-TH accumulation.

Thyroid Hormone Synthesis and Control

The thyroid follicular cell traps iodide (I) from the serum for use in synthesis of thyroid hormones. The iodide concentrated in the thyroid follicle cell is oxidized by a peroxidase at the cell-lumen interface forming oxidized species of iodine, which are incorporated into the tyrosyl residues of thyroglobulin. In the thyroglobulin, the iodotyrosines (MIT and DIT) are coupled by peroxidase mediation to form T3 and T4 (Edwards, 1983).

The synthesis and release of thyroid hormones is regulated by thyroid stimulating hormone from the anterior pituitary gland. TSH is a glycoprotein consisting of alpha and beta subunits which are linked by non-covalent bonds. The alpha subunit is common to three other glycoprotein hormones (LH, FSH and HCG) but TSH has a distinctive beta subunit which confers specificity for its given receptor. The rate of TSH release by the pituitary is determined by a balance between the negative feedback effects of plasma T4 and T3 concentrations and a positive hypothalamic stimulus through TRH. A fall in serum TH stimulates TSH release from the pituitary gland, whereas increases in serum TH lead to suppression of TSH release. An increase in either T3 or T4 leads to decreased TSH release but the T4 required is about 10X the T3 required. This is because the conversion of T4 to T3 within the pituitary gland by 5'monodeiodination (Larsen and Frumess, 1977) accounts for a significant amount of the T3 that binds to pituitary receptors. In vitro studies suggest that nuclear binding of T3 initiates the inhibition of TSH release following either T4 or T3 administration (see review by Larsen and Silva, 1983). Hypothalamic control of TSH secretion involves TRH release into the hypophyseal portal system by peptidergic neurons. TRH in turn is likely to stimulate release of TSH by increasing the intracellular calcium of the thyrotropic cells (Larsen and Silva, 1983).

TSH affects many aspects of thyroid function. It stimulates growth of the gland (Robinson et al., 1976), transport of colloid into the follicular cells, production and release of T3 and T4 to the serum, and increased uptake of I from the serum into the TG (McNabb et al., 1986). The regula-

tory action of TSH on the thyroid cell is mediated by the generation of cAMP, the second messenger "activator" of protein kinases (Scanes, 1986). A second intracellular messenger system in the thyroid may function through cyclic guanosine monophosphate (cGMP) in regulating cellular metabolism. cGMP has been found in thyroid tissue and was associated with stimulation of protein synthesis and RNA production within the gland (Degroot et al., 1984).

Measurement of TSH

When testing the actions of TSH, most studies of avian thyroid function have utilized heterologous mammalian TSH preparations, which are biologically active in birds. Avian TSH preparations when available, have been contaminated with significant amounts of LH (Gooden and Scanes, 1975). Although more recent modifications in the technique of purification have produced better preparations of avian TSH, their very limited distribution within the research community has precluded widespread use in experiments or in the production of an avian TSH-RIA. The lack of an RIA for avian TSH measurement (mammalian TSH-RIAs are not satisfactory for avian TSH measurement due to poor cross reactivity; unpublished work McNabb laboratory) has led to the use of bioassays to measure avian TSH. They typically measure TSH response utilizing the TG response (radiophosphorus uptake, Lamberg, 1955; radioiodine uptake, Shellabarger, 1954; serum T4 increase, MacKenzie, 1981; McNichols and McNabb, MS in prep) of young nestling birds standardized against the effects of different concentrations of mammalian TSH.

Thyroid Hormone Release

The process of thyroid hormone secretion involves the formation of pseudopods of the cell apical membrane, which surround a portion of colloid, pinching it off to form a colloid droplet (DeGroot, 1984). Inside the cells, the droplets fuse with lysosomes forming phagosomes in which the thyroglobulin is degraded; T4 and T3 are then released to the serum (DeGroot, 1984).

Mechanism of Action of Thyroid Hormones and Receptor Characteristics

Thyroid hormone enters the cell and binds to cytosolic proteins, the complex then enters the nucleus and binds to receptors; or TH may bind directly to the nuclear receptor with no intermediate cytosolic binding. The hormone binding to the nuclear receptor is T3, which is derived from the blood directly, or from monodeiodination of T4 within the cell. The presence of a specific T4 receptor in birds or mammals has not been documented. Saturation binding experiments with T4 have been used to examine whether a specific T4 receptor exists. This method led to the report of T4 binding sites in chick embryos (Bellabarba and Lehoux, 1985). However, the characteristics of the binding sites are more typical of a high capacity low affinity binding protein than of a "true" receptor. Thyroxine has been shown to interact with the T3 receptor, but with a lesser affinity for the receptor than T3. In quail, T4 has been shown to have 23% of the affinity of T3 for hepatic receptors (Weirich and McNabb, 1984). The nuclear binding proteins in mammals have been reported to have an afffinity for T3 that is 4-10X greater for T3 than for T4 (Surks and Oppenheimer, 1977). Most studies of thyroid hormone receptor binding characteristics have focused on hepatic nuclei because of greater receptor numbers present. In chick embryos, liver nuclei contained 1.5X the numbers of receptors found in the lung and 2X the number found in the brain (Bellabarba and Lehoux, 1985).

Physiological Actions of Thyroid Hormones

Thyroid hormones are essential for normal growth and development. Thyroid hormones may influence growth by an indirect effect involving permissive effects with growth hormone (Scanes, 1984), in addition to separate effects directly attributable to TH. Thyroid hormones are necessary for normal growth and development in birds. Thyroidectomy or treatment with thyroid inhibitors results in reduced metabolic rate, weight gain, and limiting of the growth of long bones (quail: Howrath and Marks, 1973; Singh et al., 1968). In adult animals, TH control the metabolic activity of many tissues through influences on the metabolism of carbohydrates, proteins and lipids. Thyroid hormones exert effects on carbohydrates through potentiation of the effects of insulin and the promotion of glycogen synthesis. Lipid metabolism is stimulated by TH, in the euthyroid animal especially the synthesis of lipids through control of lipogenic enzymes such as malic, acetyl coA and fatty acid synthetase. At high TH levels there is mobilization of lipid reserves. Moderate levels of TH stimulate protein synthesis at the nuclear level by regulating RNA synthesis or at the ribosomal level by affecting translation of mRNA (Bernal and DeGroot, 1980).

Hormone Turnover

There are a number of pathways by which T4 is degraded or excreted, including deiodination, deamination and decarboxylation to form to form the metabolically active forms T3 and tetraiodothyroacetic acid (TETRAC) and other unreactive degradation products such as reverse-T3 and diiodothyronines (Braverman et al., 1980). In addition, glucuronid or sulfate conjugation of the phenolic hydroxyl group leads to biliary or urinary excretion (Hutchins and Newcomer, 1966). The turnover of thyroid hormones is very rapid in birds compared to mammals with half-life of between 4-8.3 hours depending on the species tested (see review of Wentworth and Ringer, 1986). Japanese quail and Ring doves have been found to have comparable (9.5 and 10.3 hours) T4 half-lives when they were fed the same dietary I content (McNichols and McNabb, MS submitted). In

the same study, Ring doves fed a diet with lower I content showed markedly greater T4 half-life presumably due to the greater recycling and conservation of hormonal I. This indicates the importance of quantitation of dietary I to allow comparison between species in the handling of hormone degradation.

Influence of Low Iodine Availability on Thyroid Function

The metabolism of I in the TG is geared toward using a scarce and discontinuous supply. The thyroid follicular cells can extract I from the plasma and concentrate it in the interior of the cell and into the colloid space. This occurs by an I pump which is active at both the apex and base of the follicular cells (Degroot et al., 1984). The trapping of I by the TG can achieve a gradient between the gland and the serum of over 200:1 in birds (Newcomer, 1978). Birds have a very long retention time for I, especially under conditions of low availability of dietary I, which allows functional adjustment over a wide range of I intake (chickens: Rogler et al., 1961; quail: McNabb et al., 1985a). The consumption of very low dietary I content (< 100ug I/kg feed) may lead to decreased thyroid function in adult birds or their offspring. A low I diet in chickens has been reported to have little effect on on body weight, egg production or hatchability of the embryos (Rogler et al., 1961). In contrast, other studies have shown that low I availability to hens can depress body weight of chicks (chicken: Singh et al., 1968; quail: McNabb et al., 1985b) and increase incubation times of embryos of the hens (quail: McNabb et al., 1985b). Adult chickens also have been reported to have a trend toward lower serum T3 and T4 when fed a very low I diet (Newcomer, 1978). In a comprehensive comparison of thyroid function in adult doves on a very low (< 100 ugI/kg), or moderate (930 ugI/kg) I diet, low I birds were found to maintain serum T3 concentrations comparable to those of birds on the moderate I diet (McNichols and McNabb, MS submitted). This regulation of T3 occurred independent of the decreased serum T4 concentrations and TG-T4 content. This may indicate the stability of the metabolically active thyroid hormone, in the face of altered TG function. This may be comparable to a thyroid condition seen in human patients who demonstrate

a comparable thyroid functional pattern to low I doves. This includes a normal serum T3 along with depressed T4 and elevated TSH and TG hypertrophy and is termed a "recent hypothyroid" condition (Larsen, 1983). The etiology of this condition in humans is unclear.

Significance of This Project

In our laboratory, a major research goal is to compare the development of thyroid function and its hypothalamic-pituitary control in two avian species, precocial Japanese quail and altricial Ring doves. My work has focused on TG function and its pituitary control, as a part of the goal of investigating the sequence of events involved in the maturation of the HP-T axis during precocial and altricial development. In the embryonic/nestling study, I compared the maturational changes in TG response to pituitary control, in the two species. In addition, I extended an earlier description of the pattern of thyroid functional development from mid-incubation to about three week juvenile stages. Also, in a separate study, I examined the influence of iodine intake on thyroid function in adult doves and quail, and verified the assumption of similar thyroid status in the adults of the two species.

In quail, there is a significant maturation of TG function during embryonic life. There is histological evidence which demonstrates that organization of the TG into follicles begins on embryonic day 8 and is complete by day 12 (McNabb et al., 1972). McNabb et al. (1981) found detectable serum TH concentrations by day 10 in embryonic quail, followed by steady increases to a peak in serum TH during the perinatal period. The present study extends our knowledge of the serum pattern of embryonic quail to earlier stages, days 8 and 9, which are critical times of maturation in the P-T axis. In addition, I described the development of TG-TH content from day

embryonic day 8 through two weeks posthatching. The high TG-T3/T4 ratios I found before embryonic day 11 were correlated with measurements of TG iodine content at these stages (below the sensitivity of our assay) indicating that the high TG-T3/T4 ratio reflects preferential TG-T3 formation. This supports the speculation of McNabb (1987) that the high TG T3/T4 ratios seen in early embryonic quail are the result of low TG-I concentrations.

In quail, pituitary control of TG function begins about midway through incubation. McNabb et al. (1984b) found that maturation of thyroid-pituitary negative feedback occurs between embryonic days 9 and 10, as indicated by TG stimulation after thiourea treatment. In this study, I found that injection of exogenous TSH results in increased serum T4 in day 9 embryos, but not in day 8 embryos. The day 8 embryos show an increase in TG-cAMP content in response to TSH injection, indicating TSH receptor binding and second messenger production. Therefore, in quail the maturation of the TG-TSH receptor and its link to a physiological response (serum T4 release) occurs between embryonic days 8 and 9. There is also low but detectable TSH in the pituitary gland of embryos at 8 to 10 days of incubation, indicating potential for endogenous TSH release at this time. Work in chicken embryos suggests that endogenous TSH release is important in TG function during the last third of incubation in that precocial species.

The development of thyroid function in Ring dove embryos and nestlings was assessed by serum TH concentrations, TG-TH content, TG responsiveness to exogenous TSH, and pituitary gland TSH content. In an early study, McNabb and Cheng (1985) found that thyroid function in doves was low at hatching, and that there was no evidence of a perinatal peak in serum TH or TG-TH content. However, in that study the only embryonic stage studied was one day prior to hatching. The present study verifies and extends those results, by considering a more comprehensive sequence of embryonic stages, and by providing data on TG hormone content.

My studies suggest that the pituitary-thyroid axis does not mature until after hatching in an altricial form, i.e. it is delayed considerably compared to precocial quail. In embryonic doves, this is shown by the lack of serum T4 responses to TSH injection. However, nestling doves do show

serum T4 elevations in response to TSH injection. Maturation of the P-T axis therefore occurs some time between embryonic day 14 and day 2 posthatching. Another indication of maturation of the P-T axis is the production of TSH by the pituitary gland. Embryonic doves did not have detectable pituitary gland TSH, whereas the pituitary glands of dove nestlings of 4-7 days had low but detectable amounts of TSH. This absence of detectable pituitary TSH in embryonic doves is further support of posthatching maturation of the pituitary-thyroid axis, and is in striking contrast to quail which have detectable pituitary gland TSH at embryonic stages. In dove nestlings as well as quail embryos, the pituitary gland TSH reflected the pattern of TG sensitivity to TSH suggesting a parallel maturation of the two components of the axis.

In summary, the first part of this research reveals that there is low thyroid function throughout the embryonic and perinatal period of doves. I found developmental differences in the timing of maturation, between doves and quail, in the production of TSH and maturation of the responsiveness of the TG to pituitary control through TSH release. In a pattern consistent with greater embryonic maturation of the pituitary thyroid axis, quail show serum T4 responses to TSH by embryonic day 8, and detectable pituitary TSH at that time. In contrast, doves show no detectable TSH responsiveness or pituitary TSH during the embryonic period. The TG hormone content and serum hormone measurements also reflect maturational differences in the two species. This study extends the patterns of TG-TH and serum TH in doves and quail to earlier stages than previously investigated. In quail, there is accumulation of extensive TG-TH stores (production of hormone exceeds release to the serum) during the last quarter of incubation to support the high levels of release during the perinatal period. In contrast, in doves it appears that TG hormone release parallels production, with very little hormone storage until about 10 days after hatching.

The second part of this research involved a direct comparison of TG function in adult quail and doves. This provides insight into the comparability of TG function in the two species when the thermoregulatory and growth related adjustments of the hatchling and nestling stages are completed. There appeared to be differences between quail and doves in serum TH of adults, used as a reference point for the developmental studies of McNabb et al., (1984a). However, they could

not distinguish species differences from the possible effects of differences in dietary I intake due to the possible differences in I content of the commercial diets. In the present study, the two species were fed the same diet, which allowed a more direct comparison of TG function. I found that TG function is equivalent, and serum T3 concentrations are similar in quail and doves, but that there are differences between the two species in some aspects of thyroid function. Thus doves, when compared to quail, have larger TGs, higher WT/WB ratios (4X greater, despite larger body size in doves), greater stores of I in the TGs, and tend to have higher thyroidal radioiodide uptakes. Despite these ways in which dove thyroids might seem more active than those of quail, several functional assessments suggest that quail TG are essentially equivalent in function to those of the doves; both thyroidal hormone stores and the amount of ^{125}I appearing in labelled hormones six hours after radioiodine injection are approximately equal in the two species when dietary I is the same.

Although T4, (the quantitatively predominant hormone) was present at very different concentrations in the two species, quail and doves had similar serum concentrations of T3. This regulation of serum T3 is important because T3 is thought to be the metabolically active thyroid hormone, based on the presence in birds of "true" thyroid hormone receptors for only T3 (chickens, Bellabarba and Lehoux, 1981; quail, Weirich and McNabb, 1984). In quail and doves, there seem to be similar set points for serum T3 regulation and that regulation appears to be independent of serum or thyroidal T4 availability. My turnover study of serum T4 also indicates that T4 metabolism (degradation/excretion) is similar in quail and doves on the same diet (T4 half-lives of 9.5 hrs and 10.3 hrs, respectively).

In the next part of this study, I examined the role of dietary I intake on thyroid function in adult doves. Dove TG handling of different dietary I contents could then be compared to quail (McNabb et al., 1985a). I compared the impact of differences in I availability on thyroid function in Ring doves fed a low I seed diet, with other groups of doves receiving moderate levels of I in their diet or by supplementation of their water (to approximate the dietary intake of I in the moderate I diet). The most striking (and probably the most important) result was that all the groups maintained similar serum T3 concentrations, regardless of the extent of I intake. I interpret this to mean

that T3, the metabolically active thyroid hormone is regulated despite changes in TG function resulting from changes in I availability. This regulation of T3 occurs independent of changes in the quantitatively predominant serum hormone, T4, or in thyroidal hormone stores associated with changes in I availability in the different dove groups.

An examination of thyroid dynamics in these groups of doves illustrates the adjustments in their thyroid physiology that occur in response to differences in I availability. Although the low I group can be considered euthyroid, based on maintenance of serum T3 concentrations, the TGs of these doves exhibit characteristics generally regarded to be hypothyroid. The low dietary I available to the group resulted in much smaller thyroidal I stores than those in the moderate I groups. This lower I availability in the low I group presumably limits thyroid hormone synthesis resulting in the lower TG hormone content (decreased T4, no change in T3; increased T3/T4 ratio) and lower serum T4 concentrations than those observed in the moderate I groups. Negative feedback effects on pituitary release of TSH (presumably resulting from low serum T4) are suggested by both the high rates of thyroidal radioiodide incorporation and the TG hypertrophy in the low I group compared to those with moderate I intake. The I content of the dove TGs was directly related to I availability. This pattern has also been reported to occur in quail (McNabb et al., 1985a), chickens (Newcomer, 1978), and several other avian species (see review by Astier, 1980).

Doves in the present study, like quail in the previous study of McNabb et al. (1985a), had similar serum T3 concentrations despite differences in I intake. In contrast to quail, doves on the low I diet had reduced serum concentrations of T4. The doves also showed signs of a hypothyroid condition of the TG on the very low I seed diet, i.e. TG hypertrophy, low TG-I content, low TG hormone content, high TG-T3/T4 ratio, and elevated ^{125}I uptakes.

CHAPTER TWO: EMBRYONIC THYROID GLAND FUNCTION AND PITUITARY CONTROL

II-ABSTRACT

I compared, in quail and doves, the developmental pattern of thyroid hormone (TH) content in the thyroid gland (TG) and the serum, thyrotropin (TSH) influence on TG-3′,5′-adenosine monophosphate (cAMP) content and serum TH concentrations and pituitary gland (PG) TSH content.

In embryonic quail, there is considerable maturation of thyroid function prior to hatching. TG-TH content is low but detectable in day 8 embryos; TG-TH content increases 300X between day 8 and hatching (16.5 day incubation). Pituitary TSH was detectable on embryonic day 8, with higher levels found closer to hatching. The TG of 8 day embryos responds to TSH injection by increased TG-cAMP content but the serum TH response to TSH does not mature until day 9.

Serum TH concentrations suggest that the TG is under the control of endogenous TSH from the PG during the latter part of incubation.

In doves, most of the development of thyroid function and the maturation of its PG control occur after hatching. Thus, thyroid functional development is much later in doves than in quail. TG-TH content is extremely low in embryos and nestlings up to 3 days after hatching, increases slowly in nestlings up to day 10, then increases sharply. Serum TH are very low in embryos and rise steadily in nestlings to plateau after day 8. Pituitary TSH content is undetectable in embryos and in nestlings until day 4. The TG does not respond (based on serum TH concentrations) to TSH injection through the day of hatching (day 16; mean incubation period of 16.5 days), but an increase in serum TH occurs in day 2 nestlings in response to TSH injection. The magnitude of this response continues to increase during the first week after hatching.

II-INTRODUCTION

Pituitary release of thyrotropin (TSH) is important in the control of TH production and TH release into the serum. The development of the pituitary-thyroid (P-T) axis has been studied in mammals (Fisher et al., 1977; Avivi et al., 1981) and to a lesser extent in birds (chickens: Thommes and Hylka, 1978; Thommes and Jameson, 1980; Thommes et al., 1984; quail: McNabb et al, 1984b). In chickens, the P-T axis becomes functional between embryonic days 10-13 of the 21 day incubation period (Thommes and Hylka, 1978; Thommes and Tonetta, 1979). Although it is clear from circumstantial evidence that the P-T axis in the precocial Japanese quail becomes functional before hatching, the details of the time course and sequence of maturation of the components have not been documented. Very little is known of the development of the P-T axis in altricial species of birds, such as Ring doves, although there is evidence that the final, if not most of, the maturation of thyroid function occurs posthatching (McNabb and Cheng, 1985).

The objective of this paper is to compare precocial Japanese quail and altricial Ring doves with respect to the following questions: (1) How do dove and quail TG-TH and serum-TH patterns compare during the embryonic and nestling periods? (2) When does the TG first become responsive to exogenous TSH stimulation? (3) Are changes in the capacity of the TG to respond to TSH simply a reflection of changes in the TG-TH content, or does the sensitivity of the TG change with development? (4) Does the pituitary gland content of TSH reflect the degree of maturation (function) of the P-T axis (i.e. does a lack of pituitary TSH stores limit the production and release of TG-TH)?

II-METHODS

Animals

Fertilized eggs from random bred colonies of Japanese quail (*Coturnix japonica*) were incubated at 38 ± 0.5°C, and greater than 90% relative humidity in a forced air incubator. During experiments, eggs were maintained in portable styrofoam incubators under equivalent conditions. At the end of the experiment, body weights (to 0.01 gm) were measured and the TGs were dissected free of connective tissue and weighed (to 0.01 mg). Chicks were maintained in an incubator after hatching, at thermoneutral temperatures appropriate to their ages (as defined by Freeman, 1967). Water and feed (commercial game bird diet, Big Spring Mill, Elliston, VA) were available ad libitum.

Dove embryos and nestlings were obtained from our adult Ring dove (*Streptopelia risoria*) colony, which consists of individually housed breeding pairs that incubate the eggs and rear the nestlings. Dove embryo measurements were as described above for quail; in addition the weight of crop contents of dove nestlings was obtained and subtracted from total body weight.

Thyroid Gland Hormone Content

To determine TG-TH content (method of McNabb and Cheng, 1985) the glands were dissected out, weighed (to 0.01 mg) and immediately placed on ice. After homogenization, the TGs were digested with pronase (Calbiochem-Behring, San Diego, CA), extracted in 75% ethanol, then centrifuged, and the ethanol extract was stored at -30° C until assayed by RIA.

Serum Thyroid Hormones

Blood was collected from the chorioallantoic arteries of the embryos, or from the brachial veins of dove nestlings or quail chicks, into plain microhematocrit tubes. Sampling of the day 8 embryos required that the microhematocrit tubes be heated and drawn to create a smaller opening for collecting the small sample volume. The samples were centrifuged, separated and the serum stored frozen at -10° C until analysis by RIA. We used a double antibody RIA verified for use on serum of quail (McNabb and Hughes, 1983) and doves (McNabb et al., 1984), with an accuracy of 95% for T4 and 96% for T3 in relation to a euthyroid control serum (Ortho RIA Control Serum 1, Ortho Diagnostics, Raritan, NJ). Precision tests indicated that ± 2SE was 3.1% of the mean for T4 and 2.6% of the mean for T3; the lower limits of assay sensitivity were 1.25 ng/ml for T4 and 0.125 ng/ml for T3 (McNabb and Hughes, 1983). Embryonic serum samples were spiked with an aliquot of a serum standard to shift the values to a linear region of the standard curve. The added spike was tested for recovery from samples (T3 = 98%, T4 = 96%).

Thyroid Gland Response to TSH: TH Release

For evaluating the thyrotropin stimulation of the TG we used bovine TSH (Sigma Chemical Co., St. Louis, MO) which has been demonstrated to be effective in quail (McNabb et. al., 1984b). Avian TSH is not commercially available. TSH (25 mU in 10ul of 0.9% NaCl), or the equivalent amount of vehicle, was injected subcutaneously from microliter syringes (50 ul, Hamilton Co., Reno NV, fitted with 30 gauge needles). To allow injection of the embryos in the shoulder region, a small opening was made in the blunt end of the egg, followed by a small incision in the chorioallantoic membrane. Preliminary studies indicated optimal sampling times (plateau of response) to be 30 minutes (cAMP) or 60 minutes (serum TH) after TSH injection. Thyroid glands also were collected for measurements of TH content.

Dove embryos were injected with TSH or saline as described above for quail, then returned to their nests, which were transferred to an incubator at 35° C. Blood samples were collected from embryos and nestlings from day 14 of the 16.5 day incubation period through day 15 posthatching.

Thyroid Gland Response to TSH: cAMP Production

To determine cAMP content by RIA, the TGs were homogenized (on ice) in 100ul of 0.1N HCl with 0.2mM isobutylmethylxanthine (Sigma Chemical Co., St Louis, MO), a phosphodiesterase inhibitor. Extracts were centrifuged (11,000g for 5 mins) and the supernatant was separated and frozen (at -10° C) until analysis. The cAMP RIA (modified from Ferrendelli et al., 1977) made use of ^{125}I-succinyl cAMP (New England Nuclear, Boston,MA) as tracer, anti-cAMP primary antibody (Research Products International, Mount Prospect, IL) and activated charcoal separation of the bound and free antibody. The samples and standards were succinylated (addition of 4 ul of acetic anhydride and 6 ul dimethylamine) to increase the sensitivity of the assay (Harper and Brooker, 1975). Assay sensitivity for cAMP was 0.1 pmol/ml; a precision test indicated that $\pm$ 2SE was 4.9% of the mean (n = 10).

Pituitary TSH Content

There are reports of the successful measurement of serum TSH of adult Japanese quail by use of heterologous human TSH-RIA (Pharmacia Diagnostics; Almeida and Thomas, 1981; Almeida 1982) Our testing of three commercial mammalian TSH-RIA kits (New England Nuclear, Boston, MA; Diagnostic Products Corp., Los Angeles, CA; Cambridge Medical Diagnostics, Inc., Billerica, MA) showed little cross reactivity with either serum or pituitary gland extracts of normal or hypothyroid quail. Therefore, pituitary content of TSH was measured by a bioassay. Pituitary glands were dissected out after removing the top of the skull, weighed and homogenized in phosphate buffered saline (0.9%) while kept on ice. After centrifugation, the supernatant was re-

moved and stored at -30°C until assayed. Aliquots of standard concentrations of bovine TSH or pituitary homogenate samples were injected (s.c.) into 4 day quail chicks. All assays were in triplicate. Blood samples, collected from the jugular vein 120 minutes after TSH injection, were centrifuged and serum was stored frozen at -30°C until assayed for T4 as described above. The TSH content of the pituitary homogenate, was determined using a standard curve of the serum T4 response of chicks receiving known quantities of bovine TSH. Multiple dilutions of each pituitary homogenate were tested to facilitate assessing the sample in the linear region of the standard dose curve (0.1 to 1.5 mU) bovine TSH.

Thyroid Gland I Content

To determine whether the high T3/T4 ratio in TGs in quail embryos before day 13 was due to a relative I deficiency within the TGs, glands were collected from day 9 to 12 of incubation. Thyroidal I content of glands digested in perchloric acid was determined by a spectrophotometric assay utilizing decolorization of ceric to cerous ions (based on a commercial method by Hycel, Inc., Houston, TX as modified by McNabb et al., 1985). Precision of the technique for thyroid glands was 11.1% ($\pm$ 2SE expressed as a % of the mean).

Statistical Analysis

Statistical comparisons were made by Analysis of Variance and Scheffe's Multiple Comparison Method (Zar, 1986). Values of $p < 0.05$ were considered to be indicative of statistically significant differences.

II-RESULTS

Thyroid Gland Hormone Content

Embryonic quail show a progressive rise in TG-T4 content beginning at day 8 (Figs. 1 and 2). However, the rate of increase in TG-T4 with time was curvilinear. The TG-T4 content increases 2.4X between embryonic days 8 and 11, whereas there is an increase of ~ 20X between days 12 and 15. The TG-T3 content, unlike that of TG-T4, does not begin to rise until embryonic day 12. Before day 12 the TG T3/T4 ratios tend to be high, but variable (data not shown). After day 12 the more rapid increase in TG-T4 compared to TG-T3 results in a continuing decrease in TG-T3/T4 ratios until hatching (Fig 2). Following hatching, the TG-TH content remains relatively high but gradually declines toward lower adult levels; the TG-T3/T4 ratio remains low.

In doves, during the embryonic and perinatal periods the TG hormone content is very low (Fig 3), and strikingly different than that of quail (Fig 2) at comparable ages. TG-TH levels are low until nestlings reach 8 days of age then a rapid increase (4-8X) occurs in both TG-T4 and TG-T3 from days 8 through day 16 of nestling life (Fig 3). The TG T3/T4 ratios of doves are high until embryonic day 14 when a sudden decrease in the ratio occurs. The ratios remain low for the remainder of the embryonic period and in nestlings.

Serum Thyroid Hormone Concentration

The serum T3 and T4 concentrations of doves remain very low through the embryonic period (Fig 4), with no peak in serum TH concentrations near hatching, as has been observed in precocial avian species. (Quail serum TH data for a developmental sequence are not presented here because birds in this study showed a pattern identical to that presented in earlier papers from our laboratory;

see McNabb et al., 1981, 1984a). The serum TH concentrations in doves during the perinatal period are comparable to those of day 11 quail embryos; in nestlings, the serum TH concentrations gradually increase to reach a plateau by about day 13.

Response to TSH

Quail embryos show a thyroidal cAMP response to TSH at the earliest embryonic age tested (day 8; Fig 5). This responsiveness was not translated into a physiological effect in the form of TH release, i.e. there was no increase in serum T4 or serum T3. On day 9, TSH stimulation of the TG resulted in an increase in TG-cAMP content as well as an increase in serum T4 concentration compared to saline-injected embryos.

Embryonic doves did not show a serum T4 response to exogenous TSH stimulation (Fig 6; TG-cAMP was not tested because of the limited numbers of animals available). After hatching, all nestlings tested (ages 2 to 14 days) responded to TSH injections by increases in serum T4 concentrations. Several nestlings were sampled before, and 30 minutes after saline injection to verify that the results presented for TSH were not due to the injection process.

Pituitary Content of TSH

Quail had low but detectable amounts of TSH in their pituitary glands by embryonic day 8 (Fig 7) and higher amounts of TSH on day 13 and later (in 2 of 3 individuals tested). Embryonic doves did not have detectable TSH in their pituitary glands. Dove nestlings 4 to 7 days old had low but detectable pituitary TSH content (Fig 6), which corresponded to quail embryos at days 8 to 10 of incubation.

Thyroid Gland I Content

The I content of pooled TGs of day 9 to 11 embryonic quail were below detection (< 0.025 ug I/ml). The glands of day 12 embryos contained 0.15 ug I/TG pair.

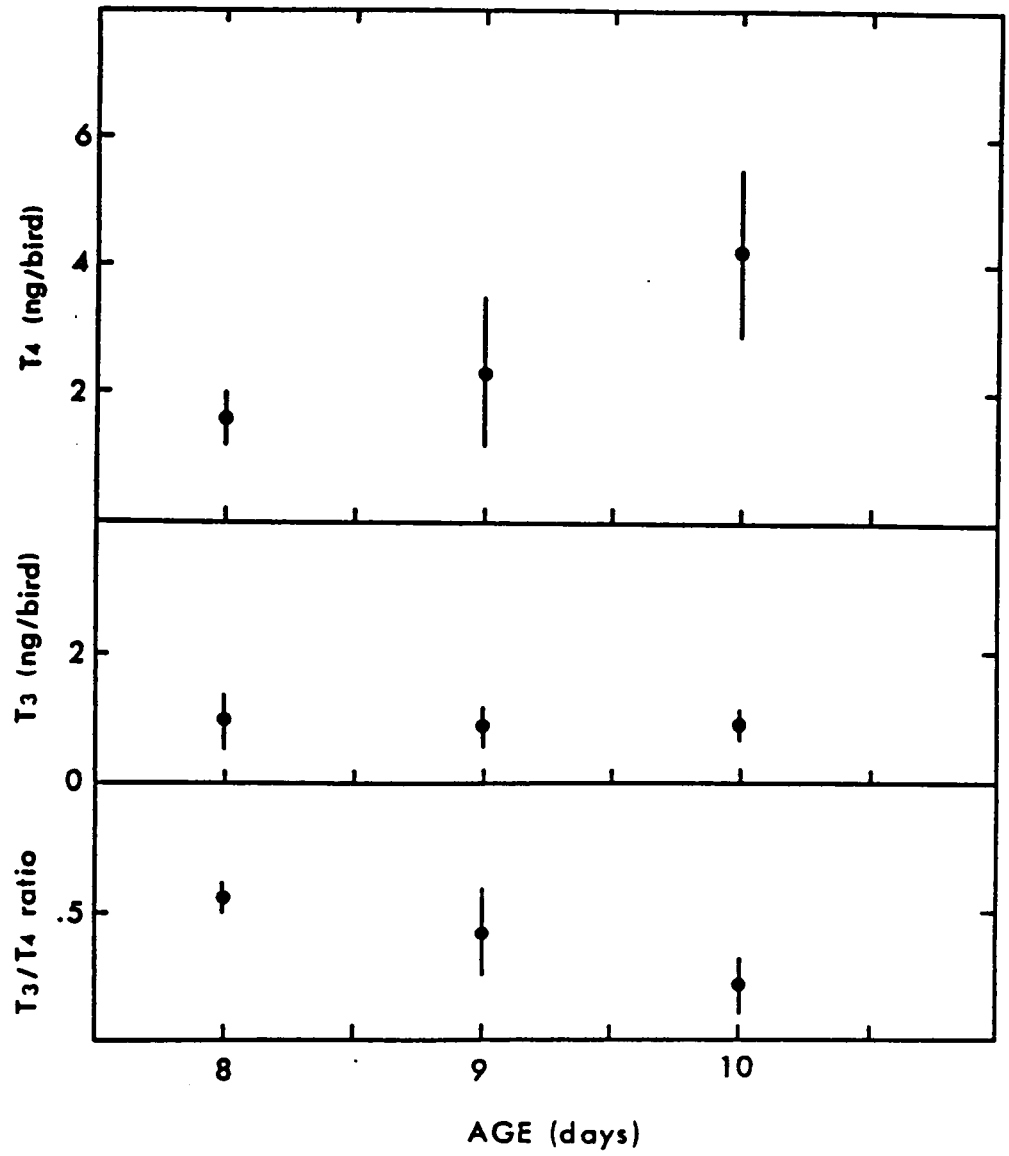

Figure 1. Thyroid gland hormone content in embryonic quail. Values are the mean ± 2SE. N = 8 per stage.

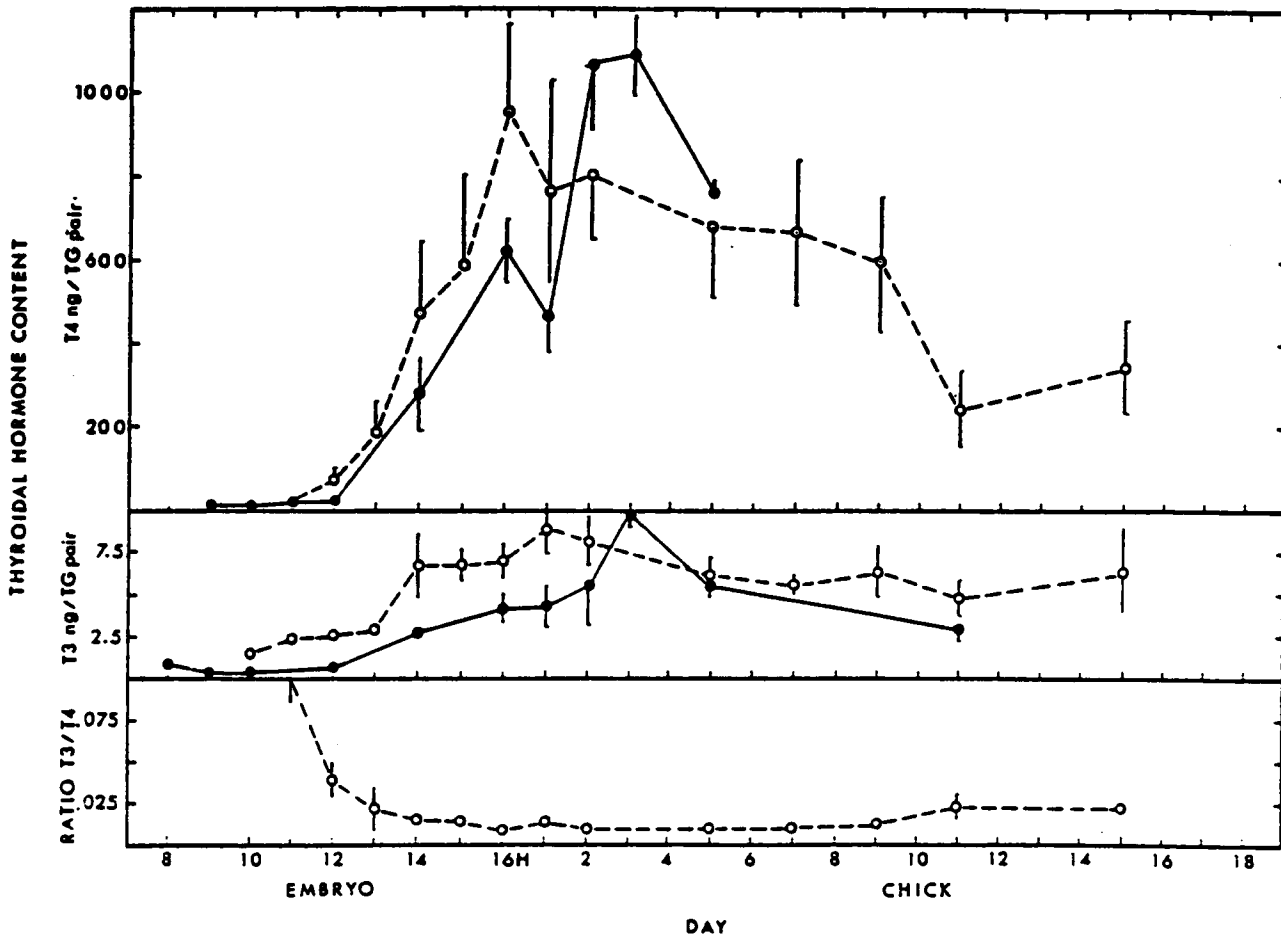

Figure 2. Thyroid gland hormone content of embryonic and hatchling quail. Open circles represent collection sequence 1, N = 6 per stage; filled circles represent collection sequence 2, N = 6-8 per stage. The T3/T4 ratio line for sequence 2 overlaid the sequence 1 line and was omitted for clarity. H designates the day of hatching. Values are the mean ± 2SE.

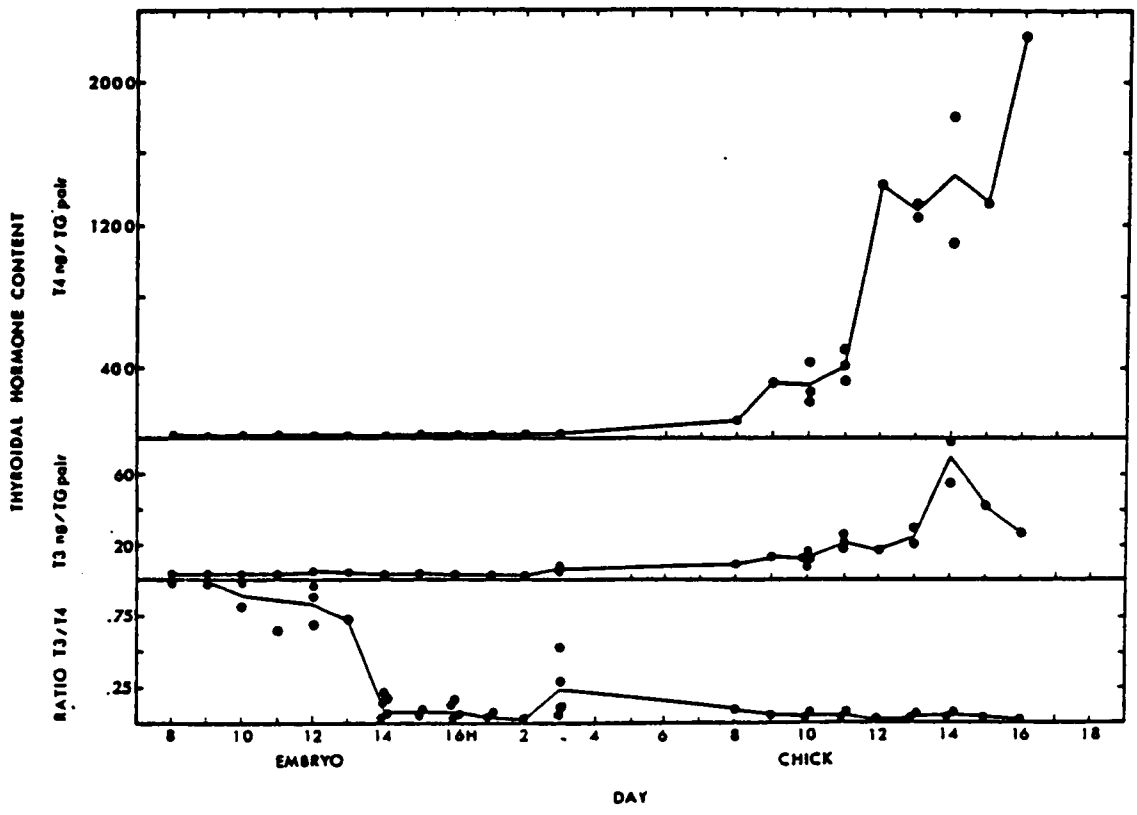

Figure 3. Thyroid gland hormone content of embryonic and nestling doves. Values represent individual birds, with a line connecting the mean value at a stage. H designates the day of hatching.

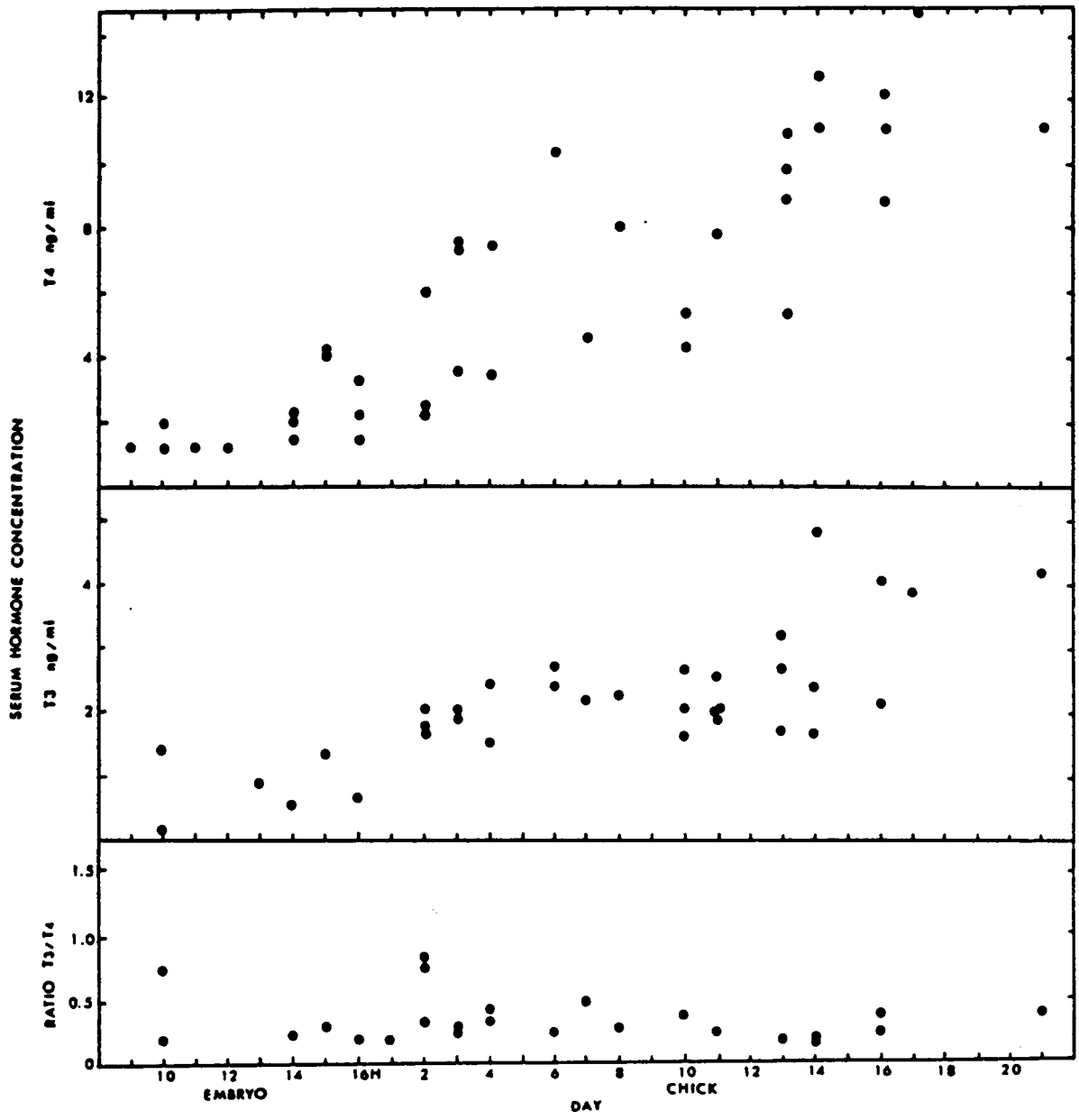

Figure 4. Serum thyroid hormone concentrations of embryonic and nestling doves. Values represent individual birds. H designates the day of hatching.

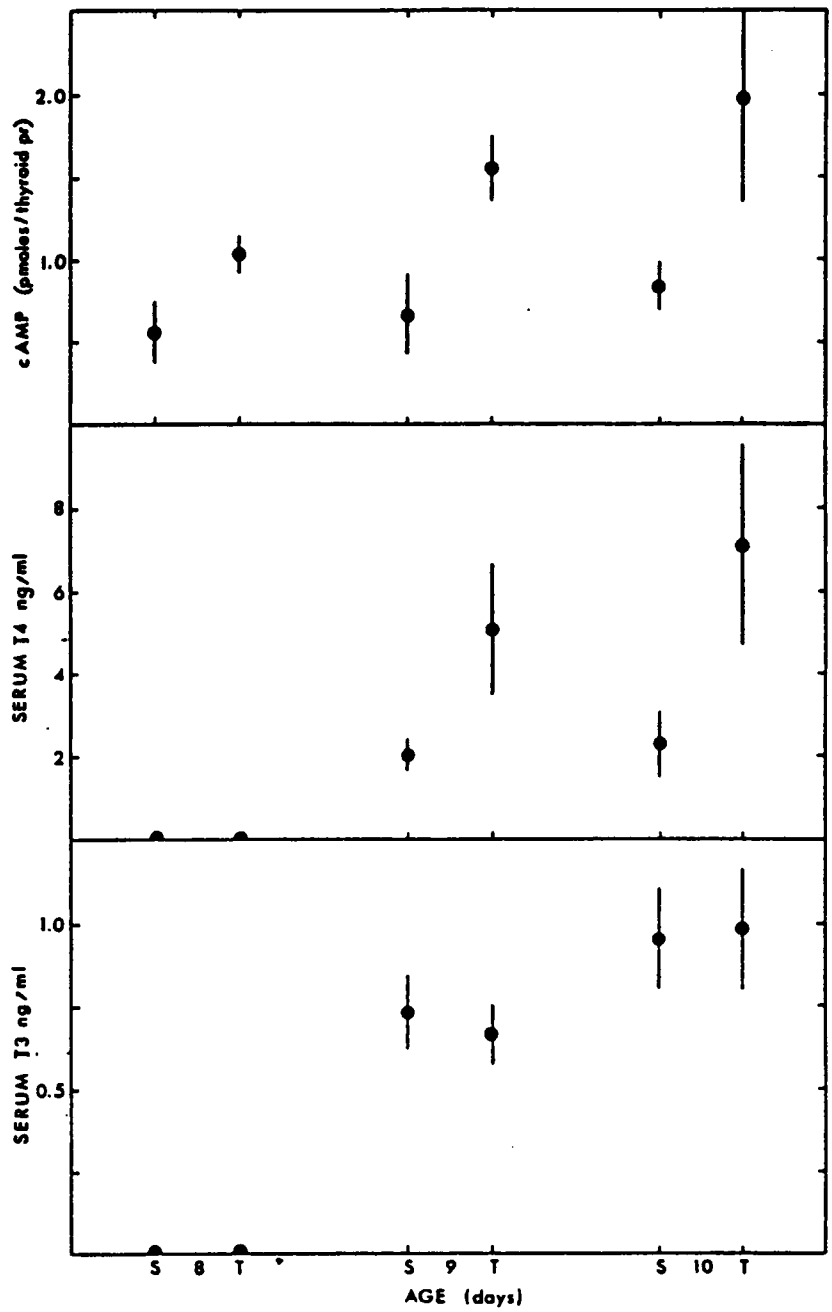

Figure 5. Serum thyroid hormone concentrations and thyroid gland c-AMP content in embryonic quail after TSH (T; 25 mU) or saline (S; 0.9% NaCl) injection. N = 10-12. Values are the mean ± 2SE.

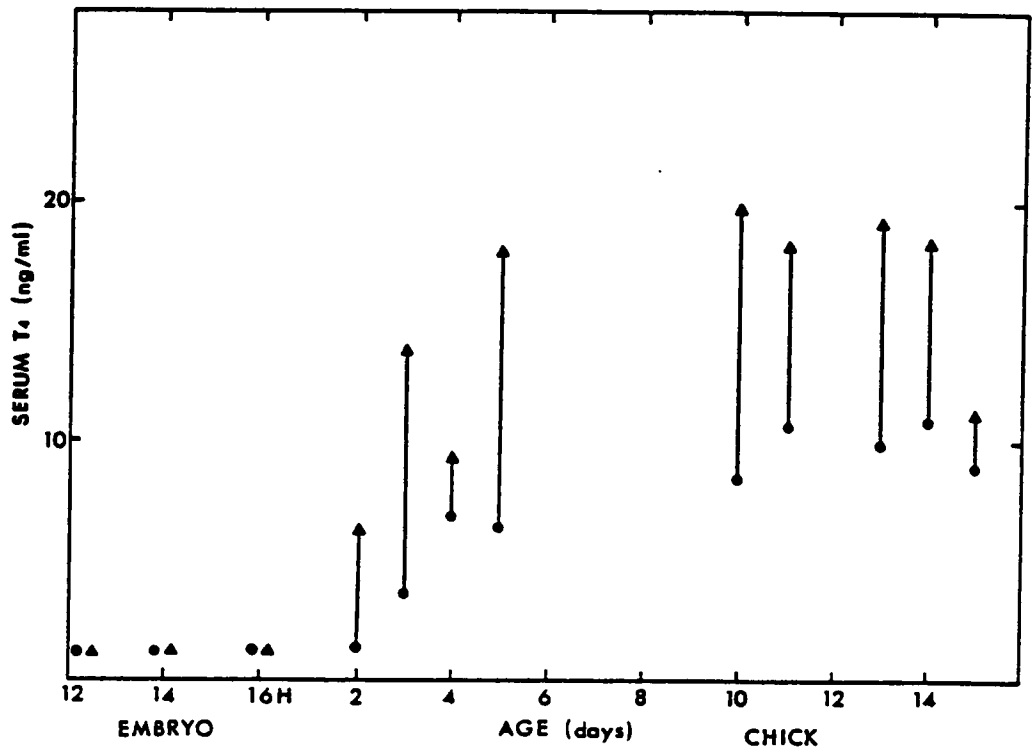

Figure 6. Serum T4 concentrations in embryonic and nestling doves at the beginning of the experiment (circles) and 60 minutes after injection of 25 mU TSH (triangles).

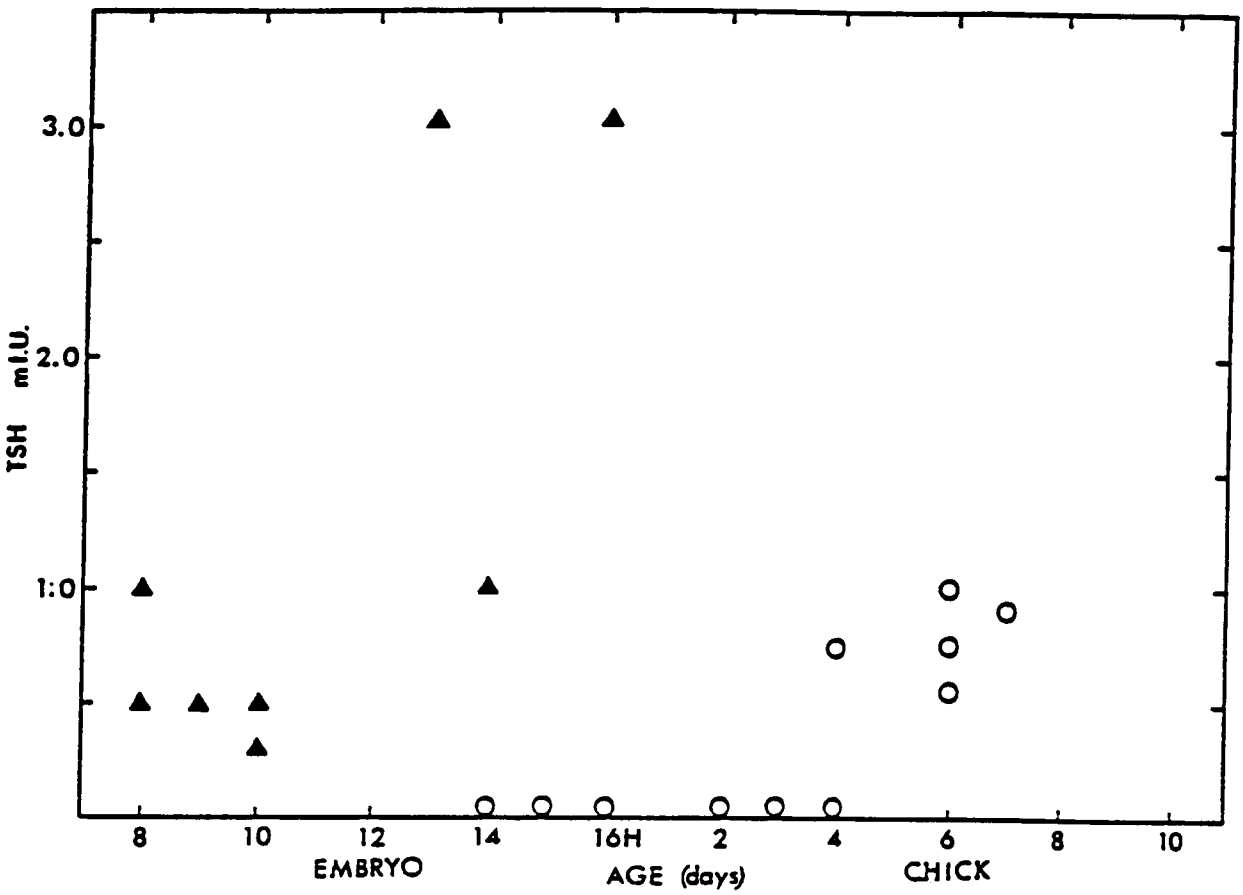

Figure 7. Bioassay of pituitary TSH content of embryonic quail (triangles) and embryonic and nestling doves (circles). See methods for details of the bioassay.

II-DISCUSSION

Hormone Content of the TG, and Serum Hormone Patterns

The relationship between TG-TH content and the serum TH pattern during development differs between quail and doves. In quail, the greater than 300 fold increase in the TG-T4 content between day 8 and hatching, reveals the accumulation of abundant TG hormone stores in late embryonic life. These stores support the surge of T4 release to the serum that occurs in precocial embryos during the perinatal period (quail: McNabb et al., 1981, chickens: Davison, 1976; Thommes and Hylka, 1977) and maintain the relatively high posthatching serum TH concentrations of chicks (McNabb et al., 1984a). The high TG T3/T4 ratio observed in embryonic quail at mid-incubation may reflect the very low TG iodine stores prior to day 12. This relationship of high T3/T4 ratio with low iodine availability also has been reported in embryonic chickens (Daugeras et al., 1976) and adult quail (McNabb et al., 1985b). In embryonic quail, our results show that TG-T3/T4 ratios begin to decrease early, i.e. day 10 embryos have significantly lower TG-TH than day 8 embryos. McNabb (1987) has suggested that the high TG T3/T4 ratios until day 11 are the result of low TG-I concentrations that lead to greater production of T3 (the less iodinated hormone) compared to T4. In the present study, we found low TG-I stores in embryonic quail at these stages. This finding is consistent with, and extends to earlier ages, the TG-I measurements of McNabb et al. (1972), and supports the idea that the high T3/T4 ratios result from low I stores. At these early embryonic stages, I availability to the TG may be limited by the lack of sufficient circulatory transport of I from yolk stores. Alternatively, a low rate of pituitary TSH release may result in low I transport from the blood into the TG because the transport rate of the I pump generally is considered to be TSH dependent (Pierce, 1974). This is supported by studies of TG-^{125}I uptake rates at these stages (McNabb et al., 1981).

In quail embryos, the serum T4 concentrations are low until about day 10, followed by a linear increase through day 15. This is followed by a dramatic increase in the serum T4, with a peak occurring close to hatching (McNabb et al., 1981). During the early embryonic period the serum concentration of T3 remains relatively stable (Fig 1). A rapid rise in serum T3 concentration begins on day 15, T3 and T3/T4 ratios peak during the perinatal period and relatively high T3 concentrations continue into the posthatching period (McNabb et al., 1981).

In contrast to quail, embryonic doves store little TG-TH and have low serum TH concentrations that increase only slightly in the last two days of incubation. Thus, the embryonic serum TH measurements of this study support, and extend to earlier stages, the pattern described by McNabb and Cheng (1985) for nestling doves (low, gradually increasing posthatching serum TH concentrations with stabilization at about adult values by days 6 to 8). The increase in the serum concentrations of T3 and T4 precedes the period when there are marked increases in TG stores of both hormones (this study, and McNabb and Cheng, 1985) Thus, it appears that hormone release parallels hormone production, with very little hormone storage until about 10 days after hatching, followed by a period that favors TH accumulation in the TG.

There also are marked contrasts between the precocial and altricial patterns of serum TH concentrations in these species. In perinatal doves, the serum T4 concentrations are as low as those of embryonic quail on day 11 of incubation. There was no evidence in doves of a perinatal peak in serum hormones such as occurs in precocial species (chickens: Thommes et al., 1977; Decuypere et al., 1979, 1982; quail: McNabb et al., 1981, 1984a). McNabb et al. (1984a) have speculated that the differences in the two patterns of perinatal TH concentrations is related differences in the timing of initiation of thermoregulatory responses in precocials and altricials. After hatching, the serum TH concentrations of doves gradually increase to reach a plateau at about day 12.

Thyroidal Sensitivity to TSH and Pituitary Gland TSH Content

Quail embryos responded to TSH, by increased TG cAMP production, at the earliest embryonic age tested (day 8). This responsiveness indicated that TSH was binding to receptors and triggering second messenger (cAMP) production. However, at this age there was no elevation in serum TH, indicating incomplete maturation of the TG link between hormone to receptor binding and hormone release. TSH stimulation of the thyroid on day 9 resulted in both TG-cAMP elevation and a serum T4 increase, indicating maturation of the linkage. These results indicate that TSH binding to receptors and cAMP generation precedes, by at least one day, maturation of the process of hormone release from the TG. However, the low TSH content of the pituitaries of early embryos, and the serum TH concentration pattern at these ages, may indicate that this lag may is not functionally important. The TG appears to be releasing small amounts of hormones irrespective of direct stimulation by TSH. This pattern, in which TG responsiveness to TSH precedes endogenous TSH production, also has been demonstrated in the chicken (Thommes and Hylka, 1978). The embryonic chicken thyroid is sensitive to TSH as early as day 6.5 (Thommes and Hylka, 1978), and thyrotropes are first detectable on day 6.5 (Thommes et al., 1983) of the 21 day incubation period. (This corresponds to embryonic day 5 of the 16.5 day incubation period in quail). However, in chickens the first apparent release of pituitary TSH (that influences serum TH concentrations) seems to occur several days later, between days 10 and 12 of incubation (Thommes et al., 1977; Thommes and Hylka, 1978).

The pituitary gland content of TSH and the the pattern of TG responsiveness to TSH mature in parallel in both quail and doves. Quail, which show responses to TSH by embryonic day 9, also have detectable TSH in the pituitary at that time. There is also a large increase in pituitary TSH content in quail on embryonic days 13 and 16 that correlates with the release of TSH and the perinatal surge in serum T4 associated with hatching. In contrast, the TGs of embryonic doves do not respond to exogenous TSH, nor do embryonic dove pituitaries contain detectable TSH. In nestling doves, pituitary TSH content increases between days 4 and 6. This coincides with a gradual

increase in TG-TH content and larger increases in serum TH, all indicating much later maturation of the pituitary to thyroid control in this altricial species than occurs in quail.

In summary, we have demonstrated developmental differences between precocial quail and altricial doves in the pattern of TG-TH content, serum TH during the embryonic and perinatal period, production of TSH and maturation of pituitary control of the TG. In doves, all the aspects of thyroid function and its control that were examined develop late (after hatching) while in quail these events are established in late incubation and exhibit a relatively mature state at hatching.

ACKNOWLEDGMENTS:

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CHAPTER THREE: COMPARATIVE STUDY OF THYROID FUNCTION IN ADULT QUAIL AND DOVES

III-ABSTRACT

Thyroid function was studied in Japanese quail, *Coturnix japonica* , and Ring doves, *Streptopelia risoria* , when both were fed the same dietary iodine (I; 930 μg I/kg). We also compared thyroid function in groups of doves receiving low I (< 100 μg I/kg) or moderate I (930 μg I/kg). We measured thyroid gland (TG) weight, TG stable I content, TG ^{125}I uptake, ^{125}I labelling of thyroid hormones, and serum ^{125}I thyroxine (T4) half-life. Triiodothyronine (T3) and T4 concentrations in TGs and serum were also determined.

Our results indicate that doves and quail receiving the same dietary I show similar serum T3 concentrations and TG functional states, but that there are some differences between the species in the way by which these equivalent functional states are achieved. When doves fed low dietary I

(< 100 μg I/kg) are compared to doves with moderate I intakes (930 μg I/kg), we found similar serum T3 concentrations despite reduced serum T4 concentrations and TG-hormone stores. This study demonstrates that quail and doves show similar TG function and a similar regulation of serum T3, the presumed metabolically active hormone, when dietary I availability is the same. Also, doves with low dietary I show decreases in some measures of TG function compared to doves with moderate I, but still maintain a level of serum T3 comparable to that of doves with adequate I intake. This set point regulation of T3 therefore appears to be independent of serum T4 or TG hormone stores.

III-INTRODUCTION

Ring doves (altricial) and Japanese quail (precocial) exhibit similar incubation times, but different patterns of thyroid functional development from embryonic stages to fledging (McNabb et al., 1984, McNabb and Cheng, 1985). While quail show considerable thyroid development during embryonic life and high levels of thyroidal activity during the perinatal period, doves show low activity at hatching and for the first few days of the nestling period. Most of the work on avian thyroid function has been on galliform birds, primarily chickens, Japanese quail and Bobwhite quail (see reviews by Astier, 1980; Wentworth and Ringer, 1986). Little is known of thyroid function in any other avian order, including columbiforms, to which doves belong. Although adult body weight (WB) and metabolic rate are similar in Japanese quail and Ring doves (Prosser, 1973), McNabb et al. (1984a) found differences in the concentrations of serum TH in the two species. However, in that study, they could not distinguish species differences from possible effects of differences in I intake.

The objectives of this study were to describe thyroid function in adult doves, on different levels of dietary I, and to contrast doves and quail when both were fed the same diet. We evaluated TG function by measuring TG-I content, hormone content, radioiodine uptake and incorporation of radioiodine into TH. We also investigated peripheral thyroid status by determining serum TH concentrations, and estimated the T4 half-life by injecting labelled T4 and measuring its disappearance from the serum.

III-METHODS

Animals

Adult Japanese quail (*Coturnix japonica*) and Ring doves (*Streptopelia risoria*), 6 to 12 months of age, were maintained under a 14L:10D photoperiod and provided food and water ad libitum. The availability of I was controlled to facilitate comparison of thyroid function between doves and quail (Table 1). The quail diet, a commercial game bird ration (R; Big Spring Mills, Elliston, VA.) consisted of a corn-soybean meal mixture (930 μg I/kg; analyses by Hazelton Labs America Inc., Madison, WI.) and was fed to both quail (QR) and dove (DR) groups to provide equivalent I availability to the two species. The seed diet (S; < 100 μg I/kg) fed to dove groups DS and DS + I was based on that used for the dove colony of the Institute of Animal Behavior, Rutgers University (Cheng, 1979), and consisted of a mixture of white (46%) and red (42%) millet, milo (5%) and wheat (7%). Iodine intake was supplemented in one dove group (DS + I) by the addition of KI (0.125 mg/l) to the drinking water, to approximate the I intake of the doves and quail fed the commercial ration (R). We consider R to be a moderate I diet (based on the work of McNabb et al.,1985b, with quail). Body weights (to 0.1g) were monitored to detect food or water avoidance. Thyroid gland weights (WT; to 0.01 mg.) were obtained at final sampling.

Thyroid Gland I Content

Thyroidal I content of glands digested in perchloric acid was determined by a spectrophotometric assay utilizing decolorization of ceric to cerous ions (based on a commercial method by Hycel, Inc., Houston, TX as modified by McNabb et al., 1985a). Precision of the technique for thyroid glands was 11.1% (± 2SE expressed as a % of the mean).

TABLE 1. Iodine availability in treatments.

Species	Group	Dietary I (μg I/kg)	Water I (mg I/l)
Quail	QR	R-Commercial ration (930)	Undetectable
Dove	DR	R-Commercial ration (930)	Undetectable
Dove	DS	S-Seed (< 100)	Undetectable
DoveI	DS + I	S-Seed (< 100)	I Water (0.125)

Thyroidal ^{125}I uptake

A dose of 2 μCi of carrier-free ^{125}I (Amersham Corp., Arlington Heights, IL) in 25μl of 0.9% NaCl was injected into the nape of the neck of each bird. After six hours the TGs were removed, blotted, weighed and counted in a Beckman DP 5500 gamma counter (Beckman Instruments, Palo Alto, CA).

Thyroid Gland Hormone Content

Total hormone content of unlabeled TGs was measured by RIA of ethanol extracts of TG homogenates digested with pronase (McNabb and Cheng, 1985). To examine the production of labelled thyroid hormones, radiolabeled TGs were homogenized, digested with pronase and separated by column chromatography on Sephadex G-25 Superfine (Sigma Chemical Co., St. Louis Mo.) using 0.01M NaOH and 0.01N NaH_2PO_4 solutions for elution (McNabb et al., 1986).

Serum Thyroid Hormone Assays

Blood samples were collected from the brachial veins into capillary tubes, centrifuged, and the serum frozen at -10°C until analysis by a double antibody RIA verified for use on serum of quail (McNabb and Hughes, 1983) and doves (McNabb et al., 1984a). Accuracy was 95% for T4 and 96% for T3 in relation to a euthyroid control serum (Ortho RIA Control Serum 1, Ortho Diagnostics, Raritan, NJ). Precision tests indicated that ±2SE was 3.1% of the mean for T4 and 2.6% of the mean for T3; the lower limits of assay sensitivity were 1.25 ng/ml for T4 and 0.125 ng/ml for T3 (McNabb and Hughes, 1983).

Thyroxine Turnover

A dose of 2 μCi of ^{125}I-labelled T4 (Abbott Laboratories, Inc., North Chicago, IL; < 2% T3 contamination as indicated by chromatography) was injected subcutaneously into the nape of the neck of each bird. Blood samples were collected from the brachial veins at times from 1/2 to 72 hours after injection. After centrifugation, the plasma radioactivity was partitioned into organic (presumed to be hormonal) and inorganic fractions using an anion-exchange resin AGI-X8 (Biorad, Rockville NY) to absorb the inorganic ^{125}I. This technique is one which was modified from a commercial system for measuring thyroid hormones as protein bound iodine (Hycel Inc., Houston, TX) and was previously employed in our laboratory to estimate labelled serum thyroid hormones (McNabb et al., 1981). Plasma samples were exposed to the resin for 20 minutes before separation, followed by a brief rinsing of the resin with deionized water; both supernatant plasma and resin were counted for radioactivity. Corrections were made for the different counting geometries by using fractional aliquots of the injected dose. To determine the efficiency of recovery and separation, plasma samples were spiked with labelled T4 and T3, or ^{125}I. The resin bound 84% of the ^{125}I but only 7% of the labelled T4 and 4% of the labelled T3. Thyroxine disappearance half-life for each bird was calculated from the change in plasma ^{125}I between samplings at 6 and 12 hrs after ^{125}I-T4 injection. This is comparable to the method previously used in quail by Singh et al., (1967) and incorporates the suggestion of Etta et al. (1972) to calculate T4 degradation from 3 to 12 hrs after ^{125}I-T4 injection. This minimizes the recycling of ^{125}I and therefore better represents the "true" T4 degradation rate.

Statistical Analysis

Statistical comparisons were made by Analysis of Variance and Scheffe's Multiple Comparison Method (Zar, 1986). Values of $p < 0.05$ were considered to be indicative of statistically significant differences.

III-RESULTS

The mean body weight of all doves was 156.1 ± 6.3g while that of the quail was 129.6 ± 10.4g. There were no statistically significant differences in initial WB between the dove groups, and none of the groups (quail or doves) changed in WB during the experiment (Table 2).

Quail vs Doves With the Same Dietary I; QR vs DR

Quail (QR) thyroids were significantly smaller than those of doves (DR) on the same diet; this was apparent in both the absolute WT and the WT/WB ratio (Table 2). Quail had lower I content in their TGs, 0.52 that of doves, but the weight-specific thyroidal I content (μg I/mg thyroid) was higher than that of the doves. Thyroid gland content of both thyroid hormones was comparable in the two species (Fig 8a,b). Serum T3 concentrations also were comparable, but serum T4 was lower in quail than in doves (Fig 8c,d). With the same I available, QR and DR had similar patterns of hormone turnover with approximate half-lives of T4 of 9.5 hrs and 10.3 hrs, respectively (Calculated from data shown in Fig. 9; see methods).

Different Dietary I in Doves; DS vs DS+I vs DR

In the doves, I content of the TGs reflected I availability, with DS on the lowest I diet having significantly lower TG-I content than the other two groups (Table 3). Thyroidal radioiodine uptake was also significantly greater in DS, (with the lowest I availability and the lowest TG-I content), than in the other dove groups (which did not differ from each other; Table 3). This change in TG-I

handling in DS was also demonstrated by DS having the highest percentage of radioiodine incorporation into thyroidal T3 and T4 (see results of column chromatographic separation of digested, radiolabeled TGs, Table 3).

The lower I availability in the diet and lower I content in the TGs of DS, compared to DS + I and DR, also resulted in the lowest TG-T4 content in DS; TG-T3 content did not differ among groups (Fig. 8a,b). Dietary I did not alter serum T3 in doves but serum T4 concentrations were significantly lower in DS than in DS + I or DR (Fig. 8c,d).

TABLE 2. Body (WB) and thyroid (WT) weights by diet at the time of sampling in quail and doves.

Group	Wb(g)	Wt(mg)	Wt/Wb X 10^4
DS	154 ± 13.0^a	86.9 ± 32.9^a	5.51 ± 1.8^a
DSI	157 ± 10.0^a	37.9 ± 8.6^b	2.35 ± 0.5^b
DR	151 ± 12.5^a	44.1 ± 11.7^b	2.99 ± 0.9^b
QR	129 ± 10.4^b	9.2 ± 1.5^c	0.69 ± 0.1^c

Doves, DS = < 100 µg/kg in seed diet, DS + I = 0.125 mg/l in water with seed diet, DR = 930 µg/kg in commercial ration; Quail, QR = 930 µg/kg .br in commercial ration. Values are expressed as the mean ± 2SE; within columns different letters indicate differences, $p < 0.05$.

TABLE 3. Thyroid gland (TG) I content, TG-radioiodine uptakes, and the percentage of radioiodine in TG-thyroid hormones (RAI-TH) in quail and doves.

Group	TG I μg/pr.	TG I/mg	I Uptake-cpm	% ^{125}I in TG	RAI-TH	TG Index
DS	15.7± 10.2[a]	0.2± 0.2[a]	3,548± 470[a]	80	8.96	7.2
DSI	579.2±225.5[b]	12.6± 3.9[b]	832± 546[b]	19	7.30	1.4
DR	1169.4±361.8[c]	24.9± 5.0[b]	1,765±1,240[b]	40	3.64	1.5
QR	507.0±207.8[b]	46.9±14.0[c]	1,236± 187[b]	28	5.25	1.5

Doves, DS = < 100 μg/kg in seed diet, DS+I = 0.125 mg/l in water with seed diet, DR = 930 μg/kg in commercial ration; Quail, QR = 930 μg/kg in commercial ration. Values are expressed as the mean±2SE; within columns different letters indicate differences, $p < 0.05$.

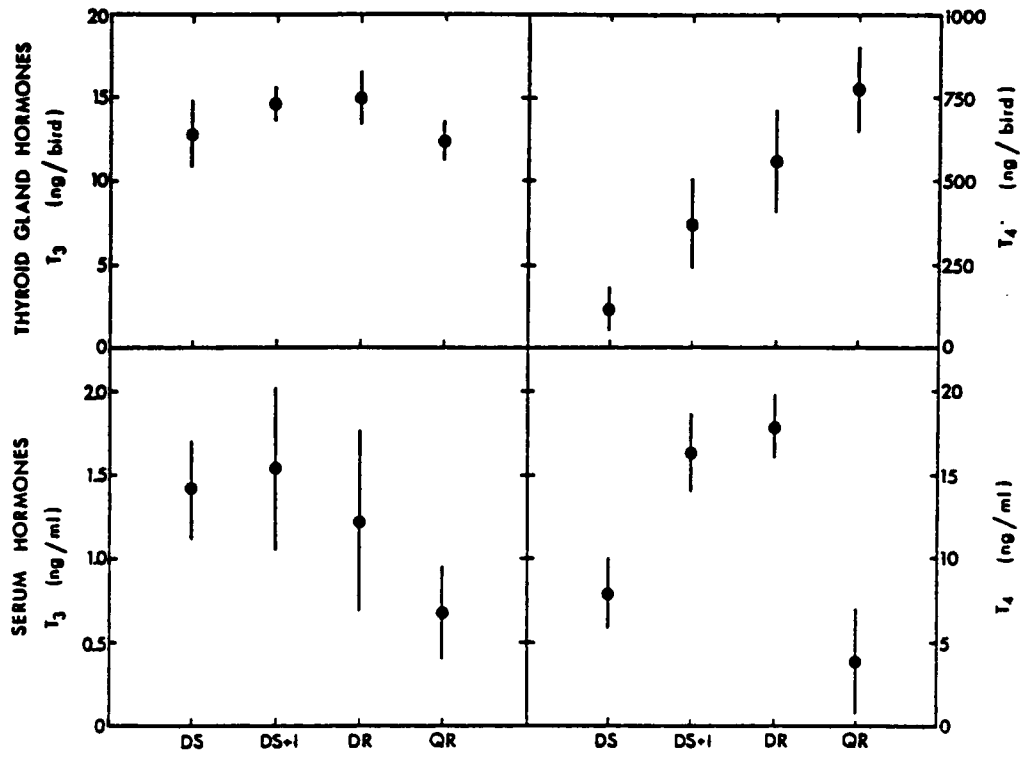

Figure 8. Thyroid gland (TG) and serum thyroid hormone concentrations of doves and quail with different I availability. Doves, DS = < 100 µg/kg in seed diet, DS + I = 0.125 mg/l in water with seed diet, DR = 930 µg/kg in commercial ration; Quail, QR = 930 µg/kg in commercial ration. Values are the mean ± 2SE (N = 8).

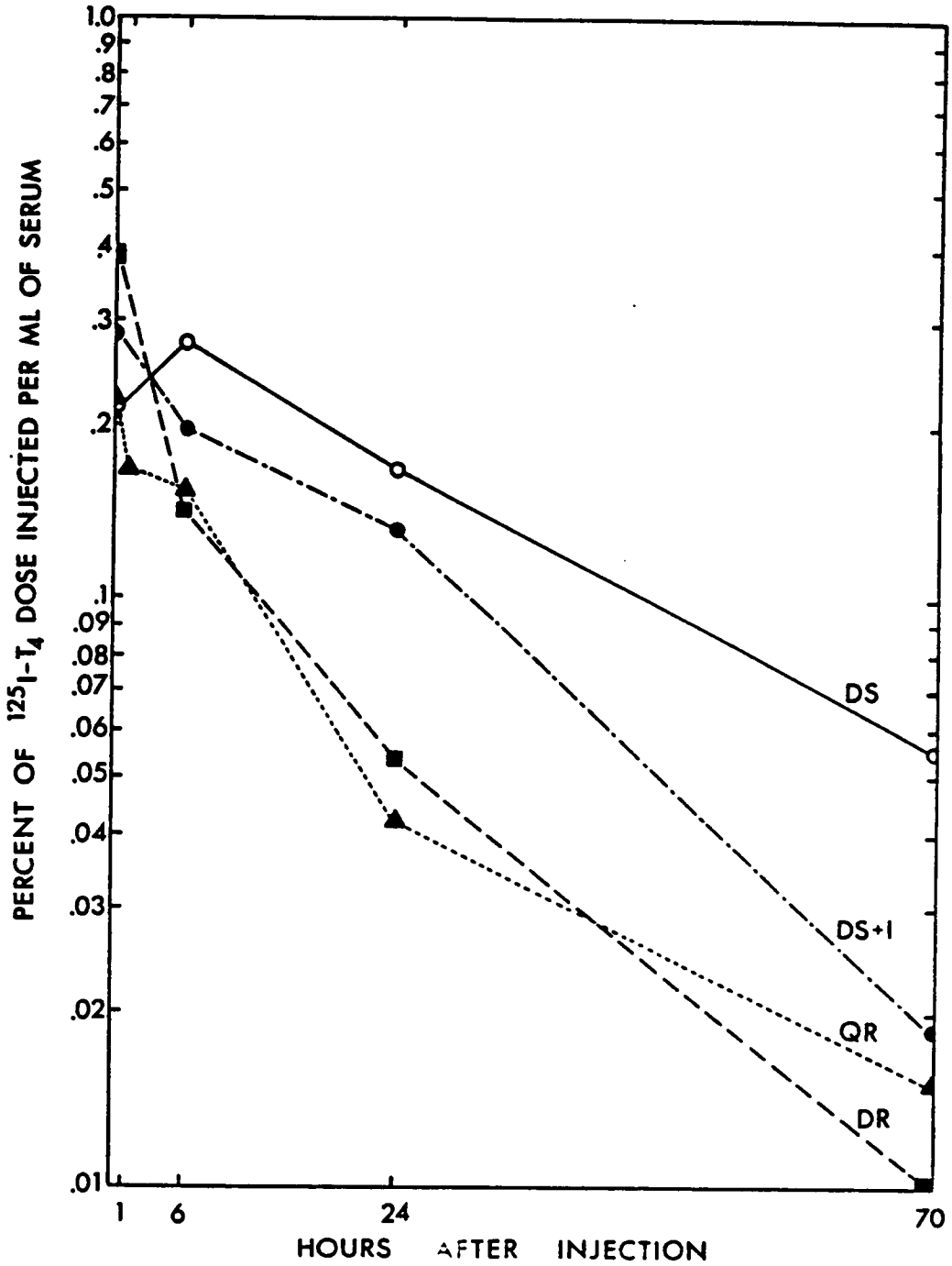

Figure 9. Post injection ^{125}I-T4 disappearance from serum of quail and doves with different I availability. Doves, DS = < 100 µg/kg in seed diet, DS + I = 0.125 mg/l in water with seed diet, DR = 930 µg/kg in commercial ration; Quail, QR = 930 µg/kg in commercial diet. Values are the mean of each group at each sampling time (N = 4).

III-DISCUSSION

Thyroid Function of Quail vs. Doves With the Same Dietary Iodide

To compare directly thyroid function in adult Japanese quail and Ring doves, we fed both species the same commercial game bird ration (groups QR and DR). Our results indicate that TG function is equivalent, and serum T3 concentrations are similar, in quail and doves, but that there are differences between the species in some components of thyroid function. Thus doves, have larger TGs, higher WT/WB ratios (4X greater, despite larger body size in doves), and greater stores of I in the TGs than quail, and tend to have higher thyroidal radioiodine uptakes. Despite these ways in which dove thyroids might seem more active than those of quail, several functional assessments suggest that quail TGs are essentially equivalent in function to those of the doves; both thyroidal hormone stores and the amount of ^{125}I appearing in labelled hormones six hours after radioiodide injection are approximately equal in the two species when dietary I is the same. Quail TGs have a functional capacity comparable to dove thyroids, because on a weight-specific basis the activity of their thyroid tissue is greater than that of doves (i.e. they have higher I content, higher T4 content and tend to have higher rates of labelled hormone accumulation after radioiodine administration, when all these variables are expressed per mg of thyroid tissue).

A comparison of the TG weights in this study with those in a previous study in our laboratory (McNabb et al., 1984a) indicates that the data for quail are comparable but doves in the previous study had much smaller thyroids (mean WT ~ 18 mg vs 44 mg in DR in the present study). In the previous study, the doves were maintained on a commercial pigeon diet, whereas, doves used in the present study had been maintained long range on a seed diet (low I) and the DR group received the game bird ration (moderate I) for a three week experimental period before sampling. Thus, it seems

likely that the relatively high WT in doves in the present study reflects some residual TG hypertrophy from their earlier low I regime.

Although T4, (the quantitatively predominant hormone) was present at very different concentrations in the two species, quail and doves had equivalent serum concentrations of T3. This T3 regulation seems to be important because T3 is thought to be the metabolically active thyroid hormone, based on the presence in birds of "true" thyroid hormone receptors for only T3 (chickens, Bellabarba and Lehoux, 1981; quail, Weirich and McNabb, 1984), as is the case in mammals (see review by Oppenheimer, 1983). In quail and doves there seem to be equivalent set points for serum T3 regulation, and that regulation appears to be independent of serum or thyroidal T4 availability. Our study of T4 half-life also indicates that T4 metabolism (degradation/excretion) is similar in quail and doves on the same diet (T4 half-lives of 9.5 hrs and 10.3 hrs, respectively).

Hormone release from the TGs could be similar in both species, and that would be consistent with our experiment showing similar TG accumulation of labelled hormones following radioiodine administration (which reflects the balance between production and release). Comparable hormone release rates could provide T4 for similar T4 to T3 conversions in the periphery and would be consistent with the similar T4 half-lives we measured and with the serum T3 regulation which seems to occur in both species. Most T3 is produced by 5′ monodeiodination of T4 to T3 in peripheral tissues in mammals (Engler and Burger, 1984) and there is considerable evidence that the same is true in birds (see review in McNabb, 1987).

Effects of Differences in Iodide Intake in Doves

In the second part of this study we compared the impact of differences in I availability on thyroid function in Ring doves fed a low I seed diet (DS) with other groups of doves receiving moderate levels of I in their diet (DR) or by supplementation of their water (DS + I; to approximate the dietary intake of DR). The most striking, and probably the most important result, was that

all the groups, regardless of the extent of I intake, maintained similar serum T3 concentrations. We interpret this to mean that T3, the metabolically active thyroid hormone, is regulated despite changes in TG function resulting from changes in I availability. This regulation of T3 occurs independently of changes in the quantitatively more abundant serum T4, or in thyroidal hormone stores associated with changes in I availability in the different dove groups. If peripheral production is the main source of T3, as argued in the previous section, even the low serum T4 concentrations in the DS group, appear to be sufficient to meet the need for T4 as substrate for T3 production.

Comparison of T3/T4 ratios shows that the ratio is higher in serum than in the TG, indicating that some monodeiodination of T4 to T3 must be occurring in all groups of doves tested (Table 4). This deiodination could occur either during the process of hormone release or in peripheral tissues. The study of Lam et al. (1986) indicates that there is no apparent conversion of T4 to T3 during hormone release in chickens, even from TSH-stimulated TGs. Thus, their study suggests that the characteristically higher T3/T4 ratios of serum compared to TGs in birds is solely due to peripheral (extrathyroidal) deiodination.

An examination of thyroid dynamics in these groups of doves illustrates the adjustments in their thyroid physiology that occur in response to differences in I availability. Although the low I (DS) group can be considered euthyroid, based on maintenance of serum T3 concentrations, the TGs of these doves exhibit characteristics generally regarded to be hypothyroid. The low dietary I available to the DS group resulted in much smaller thyroidal I stores than those in the moderate I groups. This lower I availability in DS presumably limits thyroid hormone synthesis resulting in the lower TG hormone content (decreased T4, no change in T3; increased T3/T4 ratio) and lower serum T4 concentrations than those observed in the moderate I groups (DS + I and DR). Negative feedback effects of the low serum thyroid hormone concentrations on pituitary release of TSH are suggested by both the high rates of thyroidal radioiodine incorporation and the TG hypertrophy in the DS group compared to DS + I and DR.

TABLE 4. Serum T3/T4 and thyroid gland (TG) T3/T4 ratios in doves and quail with different iodine intakes.

Group	Serum T3/T4	TG-T3/T4
DS	0.291^{a}	0.177^{a}
DSI	0.216^{ab}	0.106^{ab}
DR	0.098^{ab}	0.034^{b}
QR	0.067^{b}	0.017^{b}

Doves, DS = < 100 µg/kg in seed diet, DS + I = 0.125 mg/l in water with seed diet, DR = 930 µg/kg in commercial ration; Quail, QR = 930 µg/kg in commercial ration. Within columns different letters indicate significant differences at $p < 0.05$.

Despite some differences in thyroid function between the two moderate I groups (DR and DS + I), we believe that they can be considered together, for comparison with the low I group (DS). The similar serum thyroid hormone concentrations, TG hormone contents as well as WTs and TG radioiodine uptakes in groups DR and DS + I indicate that these groups were comparable, and had sufficient I compared to the low I of DS. Although DS + I and DR are similar for most parameters measured, there are indications that the dynamics of hormone production and turnover may differ between them, as indicated by a shorter T4 half-life, and a tendency toward higher radioiodine incorporation into TG hormones in DR than in DS + I. These trends are probably due to the greater TG-I stores in DR compared to DS + I; thus it appears that DS + I doves drank less of the I supplemented water than we had anticipated.

Comparisons With Other Studies of Iodide Intake and Thyroid Function

Quail in the present study exhibited thyroid function comparable to those on the same dietary I in our earlier study (McNabb et al., 1985). In that study, we found that dietary I above 150 μg I/kg was sufficient for "normal" thyroid function. Thus, I supplementation such as that in the commercial game bird ration used in the present study (930 μg I/kg) provides abundant I. The I content of the dove TGs was directly related to I availability. This pattern has been reported to occur in quail (McNabb et al., 1985), chickens (Newcomer, 1978), and several other avian species (see review by Astier, 1980).

Doves in the present study, like quail in our previous study (McNabb et al., 1985) had similar serum T3 concentrations despite differences in I intake. In contrast to quail, doves on the low I diet had reduced serum concentrations of T4, the quantitatively predominant serum thyroid hormone. Both species showed signs of a hypothyroid condition of the TG on the very low I diets, i.e. TG hypertrophy, low TG-I content, low TG hormone content, high TG-T3/T4 ratio, elevated

^{125}I uptakes (doves on DS, this study; quail on a purified diet containing < 50 μg I/kg, McNabb et al. 1985).

A comparison of our data on the formation of labelled thyroid hormones after radioiodine injection in doves and quail with those of Astier (1975) on ducks and rats indicates that the rate of such labelling provides an index of the functional state of the TG. Thus when a labelling index is calculated (by multiplying the % TG uptake of injected label by the % of the thyroidal label in T3 + T4), the values are inversely related to I intake; values are low in quail and doves on I-adequate diets (1.4-1.5 in QR, DR, and DS + I) and high in doves on low I diets (7.2 in DS; Table 2). Likewise, in Astier's study, ducks had a low labelling index (1.0) when fed an I-adequate diet with 870 μg I/kg and a high index (4.3) when on a severely I deficient diet. Rats also followed the same pattern; index of 1.4 when on a 600 μg I/kg diet and 8.2 when on the I deficient diet (Astier, 1975). Thus, such an index of hormone labelling seems to have value in assessing differences in thyroid activity between treatments such as those involving differences in I intake in this study. Another measure of thyroid state affected by I availability is the T4-half life. In the present study, doves with low I availability showed increased T4-half life times. This pattern also occurred in ducks fed a low I diet (Astier, 1975).

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