The Effects of Different Iodine Availabilities on Thyroid Function During Development in Japanese quail

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(ABSTRACT)

Day 14 embryos (16.5-17 day incubation period) and 1 day old chicks of Japanese quail (*Coturnix japonica*) were used to study the effects of different egg iodine (I) availabilities on thyroid function during development. Low ($\leq$50 μgI/kg feed in the maternal diet) and high (1200 μgI/kg feed) I availability were compared to control levels (800 μgI/kg feed).

Thyroid gland (TG) content of I, triiodothyronine (T3), and thyroxine (T4), plasma concentrations of T3 and T4, and hepatic 5′ monodeiodinase (5′-D) activity was measured, and the response of the TG to thyrotropin (TSH) stimulation [TG-cAMP content and plasma thyroid hormone (TH) concentrations]. Also, the developmental patterns of TH concentrations in the TG and plasma were determined.

With increased I availability, TG-I content is elevated but thyroidal T4 and T3 and the developmental pattern of TG-TH were not different from controls. Plasma T3 and T4 and the developmental pattern of plasma TH were not altered. Indicators of the TG response to TSH stimulation were not different with increased I availability. Hepatic 5′-D activity did not differ between control and high I availability. Reduced body weight was associated with increased I availability. In general, TG weight was not altered, but a small percentage of the high I birds exhibited TG hypertrophy and altered TG function.

With low I availability, TG-I content was reduced. Although thyroidal T4 content was reduced on embryonic day 14 and thereafter, TG-T3 was maintained throughout development. The magnitude of the TG response to TSH stimulation was not altered with reduced I availability. Hepatic

5′-D activity, plasma TH concentrations and the developmental patterns of plasma TH were not different between control and low I availability. Reduced I availability did not affect body or TG weight.

Developing Japanese quail exhibit excellent ability to adjust thyroid function over a wide range of I availabilities. Regulation appears to occur at TH synthesis which allows most aspects of thyroid dynamics to remain unchanged in the maintenance of circulating TH concentrations.

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Table of Contents

List of Illustrations

List of Tables

Literature Survey

I. Thyroid Hormone Effects on Growth and Development

A. Mammals

TH are required for normal development in mammals. In altricial mammals, such as the rat, which has been the subject of extensive study, most research has focused on the postnatal effects of TH (see reviews by Greenberg et al., 1974; Schwartz, 1983; Legrand, 1986). In the rat, TH are important after birth for the attainment of normal body size and weight. However, it is not known whether TH may be acting in a direct way, permissively or in concert with other hormones and growth factors. TH stimulate the production and release of pituitary growth hormone (GH), and may act synergistically with GH on tissues. In addition to overall body growth, development of many tissues is influenced by TH postnatally. Skeletal muscle mass, bone development, intestinal

maturation and kidney function are TH-dependent in the rat after birth. The central nervous system (CNS) has a critical period when TH are required for normal developmental events between 10 - 15 days after birth.

It is often assumed that most or all of TH effects are postnatal in altricial mammals. However, despite this generalization, several tissues have been found to require TH prenatally in the embryonic rat. TH deprivation late in gestation can cause irreversible damage to the CNS. TH also influence the maturation and differentiation of cartilage and lung before birth in the rat (reviews by Greenberg et al., 1974; Legrand, 1986).

In precocial mammals, such as the sheep or monkey that develop to a more advanced state prior to birth compared to the altricial rat, TH also are important for the development of most organ systems. However, the requirement for TH occurs earlier, i.e. in utero, than in altricial species (see review by Legrand, 1986). TH deprivation before birth causes decreased body weight and bone growth, reduced CNS development, and retardation in the development of wool or hair.

Developmental patterns of plasma TH concentrations are different between altricial and precocial species. In the rat, plasma TH are low at birth and rise over a three week period, before falling to adult values. In contrast, circulating TH in the sheep rise much earlier (late gestation) than in the rat, to peak just after birth (see review by Fisher et al., 1977). Thus, increases in plasma TH appear to be correlated with the time that tissues become TH-dependent with respect to growth.

Peripheral tissue TH content is regulated by plasma TH availability and by intracellular factors. Activity of 5′-D, which converts T4 to metabolically active T3, in peripheral tissues such as liver or kidney, increases at birth in the sheep, or in the first two weeks of postnatal life in the rat (see review by Kaplan, 1986). Nuclear receptor numbers in the brain also increase during the two weeks after birth in the rat. The CNS is known to be particularly TH-sensitive at this stage (Schwartz and Oppenheimer, 1978).

Thus, a comparison of altricial and precocial mammalian species reveals different temporal patterns of the effects of TH on growth and development. In addition, the tissue TH dependency for each developmental mode occurs at a time when circulating and intracellular TH are increasing.

B. Birds

In precocial avian species, such as the chicken or quail, the influence of TH on many tissues is apparent before hatching. Although hormone deprivation studies have shown that development during the first half of incubation may be TH-independent, TH deprivation during the last few days of incubation can cause decreased body weight and bone growth. Growth and maturation of skeletal muscle and cartilage also require adequate TH before hatching (see review by King and May, 1984). In addition, TH affect development of lung, duodenum, epidermal scales and feathers (see reviews by Freeman, 1974; McNabb, 1987). These effects of TH deprivation on growth are not a result of decreased GH concentrations. It is known that hypothyroid chicks have increased levels of GH, although the role of GH in avian species is still unclear (see review by Scanes et al., 1984).

TH continue to influence development after hatching in precocial birds. Body and bone growth can be reduced with TH deprivation, but the effect is less severe in chicks made hypothyroid after hatching than in those treated in ovo (see review by King and May, 1984). Gonadal function and egg production are reduced in hypothyroid chickens (see review by Wentworth and Ringer, 1986).

No studies have addressed the dependency of tissues on TH for normal development in altricial birds such as the Ring dove, the only altricial avian species in which thyroid function has been studied. However, the developmental pattern is more like that of altricial mammalian species than that of precocial birds. Ring doves hatch at a relatively less advanced stage than chickens or quail. The period following hatching is characterized by low metabolic rates, complete dependence on maternal care for heat and nourishment, and extremely rapid growth (Breitenbach and Baskett, 1967; McNabb et al., 1984). Not until approximately one week following hatching do nestlings develop a full feather coat, improved muscular coordination and, shortly thereafter, homeothermy. This pattern of development is in contrast to the precocial chicken or quail in which much of growth and maturation occurs in ovo; feather coat, muscular coordination, and the initial endothermic response are present at hatching (McNabb and McNabb, 1977).

The pattern of TG function and plasma TH also are different in altricial doves and precocial chickens or quail (see review by McNabb, 1987). Increased TG activity and circulating TH concentrations characterize the late stages of incubation (chickens: Thommes and Hylka, 1977; quail: McNabb et al., 1981) when growth and maturation/differentiation of several tissues are TH-dependent. Hepatic 5′-D activity increases during the perinatal period and may contribute to the peak in plasma T3 concentrations associated with hatching (Hughes and McNabb, 1986). Hepatic T3 nuclear receptor binding capacity increases during embryonic life in the chick (Bellabarba and Lehoux, 1981).

In contrast to this precocial pattern, altricial doves have comparatively low TG activity and circulating TH throughout embryonic life. TG function and TH concentrations rise gradually during the week following hatching, concurrent with rapid body growth and maturation of presumably TH-dependent systems. Increases in hepatic 5′-D occur post-hatching in doves but are not as well correlated with increases in plasma T3 concentrations as in precocial species (McNabb and Cheng, 1985). Additional studies are needed to determine the effects of TH on development in altricial avian species. It appears that precocial chickens and altricial doves have different patterns of TG activity as well as of TH-dependent growth.

C. Mammals versus Birds

In a comparison of TH effects on development in mammalian and avian species, there is a correspondence between species with similar modes of development. In precocial animals, such as chickens and sheep, TH stimulate general body growth and tissue maturation before birth/hatch. TG activity increases during embryonic life and TH concentrations peak during the perinatal period of precocials. In doves and rats, both altricial species, TG function does not increase until after the first weeks of postnatal life. Although nothing is known concerning TH dependency for normal development in doves, one could infer that the most profound effects of TH are likely to occur

postnatally as in the rat. Thus, the temporal differences in the requirement of TH for normal development appear to be correlated with the developmental pattern i.e. altricial versus precocial, rather than mammalian versus avian.

II. Thyroid Gland Function

A. Mammals

The TG of vertebrates is composed of follicles which are the basic functional unit. Each follicle consists of a sphere of epithelial cells surrounding a central colloid in which TH precursors are stored. The follicle is surrounded by a capillary network and so the basal membrane of the cells is in close association with the circulation.

1. Hormone Synthesis

The TG has the ability to concentrate I by greater than 100 times that found in the serum. I is trapped via a Na^+, K^+-ATPase pump located in the basal membrane, and then diffuses across the cell down an electrochemical gradient and crosses the apical membrane (see review by Utiger, 1987).

Thyroglobulin (Tg), a large glycoprotein synthesized in the rough endoplasmic reticulum (rER), is transported to the apical membrane for iodination. Thyroid peroxidase, which is associated with

the rER and is transported to the apical membrane in vesicles with the Tg, converts iodide to the iodinating species, which could be I^+, in the presence of H_2O_2 (see review by Nunez and Pommier, 1982). The H_2O_2 generating system is not fully elucidated but appears to metabolize glucose via a pentose monophosphate shunt in the reduction of NADP. NADPH is essential for the reduction of molecular oxygen and the formation of H_2O_2 (see review by Hadley, 1984). In the process of activating iodide, thyroid peroxidase and H_2O_2 also catalyze the iodination of the tyrosine residues of Tg. Iodination of tyrosine produces monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues within the Tg molecule. Recent evidence indicates that the tyrosines have different reactivities depending on the microenvironment (i.e. amino acid sequence) adjacent to them in the Tg molecule (see reviews by Taurog, 1986; Tepperman and Tepperman, 1987).

The peroxidase-H_2O_2 system also catalyzes the coupling of iodotyrosines to form iodothyronines (T4 and T3) within the Tg molecule (see review by Utiger, 1987). Two DIT residues are condensed to form T4 with the removal of an alanine group and the formation of an ether bridge between the phenyl rings of the residues. Similarly, MIT and DIT are combined to form T3 (see review by Norris, 1985). The structural configuration of Tg is important in the coupling reaction and only a fraction of the iodotyrosines will form iodothyronines. These are referred to as hormonogenic residues (see review by Marriq et al., 1985).

The level of I available during organification and coupling is important in determining the amounts and relative abundance of iodinated products within the gland. At low levels of thyroidal I, MIT content of the gland may exceed that of DIT. The same relationship exists between T3 and T4 at low TG-I content (see reviews by Nunez and Pommier, 1982; Taurog, 1986). Thus, high MIT/DIT and T3/T4 ratios are found when thyroidal I is low.

With increasing amounts of TG-I, T3 and T4 content increases to a plateau as Tg is iodinated further. Thus, it appears that there are a limited number of hormonogenic residues within Tg (see reviews by Nunez and Pommier, 1982; Taurog, 1986). Iodination of Tg also reaches a limit; the I content of Tg is approximately 0.85% in mammals (Hashino and Ui, 1970).

The incorporation of I into Tg occurs at the apical membrane and may be facilitated by the movement of Tg to reactive sites on the apical membrane surface (see review by Hadley, 1984).

Following iodination, Tg is released from the apical membrane and diffuses into the protein matrix of the colloid which fills the follicle lumen. There appear to be different pools of Tg and different turnover rates within the colloid (see review by Greer and Haibach, 1974). This may be related to the heterogeneity of the Tg molecules. The degree of iodination of Tg is variable and this affects the molecular properties of Tg during its residence in the colloid (see reviews by Ui, 1974; Kohn et al., 1985; Martin, 1985).

2. Hormone Release

Hormone release involves the absorption of stored Tg into the follicle cells, primarily under stimulation by TSH from the pituitary. Under basal conditions, some colloid may enter the cell by micropinocytosis and the most recently iodinated Tg is the first to be absorbed because of its proximity to the apical membrane (see reviews by Martin, 1985; Tepperman and Tepperman,1987). Stimulation of the gland by TSH causes the formation of pseudopods and the macropinocytosis of colloid from further in the lumen, so older, more iodinated Tg is absorbed (see reviews by Wolff and Williams, 1973; van den Hove-Vandenbroucke, 1980). Simultaneously, lysosomes migrate toward the apical membrane and fuse with the colloid droplets to form phagolysosomes. Proteolytic enzymes within the lysosomes degrade Tg resulting in the release of iodotyrosines and iodothyronines (see reviews by Wolff and Williams, 1973; Norris, 1985; Tepperman and Tepperman, 1987). T3 and T4 released from Tg probably diffuse through the basal membrane to enter the circulation generally in the same ratio as they occur in the Tg. However, intense TSH stimulation causes some T4 to be deiodinated to T3 by 5′-D within the TG cell (Ericson et al., 1982; Wu et al., 1985). MIT and DIT released during Tg degradation are deiodinated rapidly within the cell by an iodotyrosine deiodinase. Eighty to ninety percent of this second iodine pool is reused for organification of Tg (see review by Norris, 1985).

The mechanisms of TH synthesis and release have been studied extensively in mammals. The summary presented here is the general picture in mammals, resulting from work with a large number of species, of which the majority is with rats.

B. Birds

TG function in birds has received less attention than in mammals. Research with avian species has shown that TG function is similar between birds and mammals. The intracellular organelles and structures responsible for Tg production, TH synthesis, storage and release found in mammalian TG cells, are present in the avian TG (see review by Astier, 1980). Tg structure as well as its synthesis and iodination are comparable to mammals (see review by Falconer, 1971). However, there are reports of a higher degree of iodination of Tg in chickens (1.8%) compared to rats (0.5-0.85%: Hashino and Ui, 1970) This may be due to the relatively high proportion of tyrosine in avian Tg (3.4%) compared to mammals (2.1%) (see review by Astier, 1980). Iodinated avian Tg contains the same iodotyrosines (MIT and DIT) and iodothyronines (T3 and T4) as are found in mammalian TG (see review by Falconer, 1971; Astier, 1980).

III. Peripheral Thyroid Hormones in Birds and Mammals

TH are present in the circulation of both birds and mammals. However, avian species have higher T3/T4 ratios (0.1-0.5) than mammals (0.02-0.1) due to higher T4 concentrations found in mammals (see review by Astier, 1980). 5'-D is present in several tissues of birds (Hughes and

McNabb, 1986; Freeman and McNabb, 1986) and mammals (see review by Leonard and Visser, 1986) and plays an important role in peripheral T3 concentrations.

A large percentage of circulating TH are transported attached to binding proteins and it is believed generally that the free (unbound) fraction determines TH availability for entry into the cells (see review by Ekins, 1986). In most mammals, the greatest amounts of TH are carried by thyroxine binding globulin (TBG) with thyroxine binding prealbumin and albumin serving a quantitatively less important role. TBG is not present in birds; albumin and prealbumins are the primary binding proteins for TH (McNabb and Hughes, 1983; see review by Wentworth and Ringer, 1986).

Control of TG function appears to be similar in avian and mammalian species. Circulating concentrations of TH are detected in a negative feedback loop, primarily by the pituitary but also by the hypothalamus. Release of the tripeptide thyrotropin releasing hormone (TRH) from the hypothalamus stimulates pituitary release of TSH which is stimulatory to most, if not all, aspects of TG function (see review by Wentworth and Ringer, 1986).

IV. The Effects of Low Iodine Availability

A. Mammals

Studies in mammals have demonstrated that the TG has excellent ability to adapt to fluctuations in I availability. Autoregulatory mechanisms and higher control centers participate in the adjustments of TG function when I is limiting. Rats have been used extensively in the study of I deficiency (see review by Taurog, 1986).

1. Acute Effects

Acute reductions in I availability are reflected quickly in decreased TH formation and decreased TH release (see review by Studer et al., 1974). The resulting decrease in plasma TH is detected by the anterior pituitary, the major regulator of TG function. TSH, released by the pituitary in response to decreased TH concentrations, stimulates TG function. Increased TH release is initiated rapidly after TSH stimulation, followed by an increase in I uptake by the gland (see review by Studer et al., 1974).

2. Chronic Effects

With chronic I deficiency, additional functional alterations occur (see reviews by Studer et al., 1974; Taurog, 1986). Restricted I availability results in reduced TG-I and thus reduced iodination of Tg. At low I/Tg ratios, increased T3/T4 ratios have been reported due to the relative prevalence of MIT residues compared to DIT within the Tg molecules (see review by Nunez and Pommier, 1982). Increased T3/T4 ratios of hormone released from the gland are a consequence of the changes in the ratio of TH synthesized and also may reflect increased intrathyroidal T4 deiodination by 5′-D stimulated by the increased TSH (Erickson et al., 1982; Wu et al., 1985). The shift in the ratio of T3/T4 synthesized and released represents an efficient mechanisms for adapting to I deficiency. T3 contains one less atom of I than T4; T3 also is more potent metabolically.

As a result of the altered T4 synthesis and release, circulating concentrations of T4 are reduced with low I availability. T3 concentrations often are unchanged with low I, unless the shortage is severe and uninterrupted. The result is an increase in the T3/T4 ratio in the circulation which reflects the altered TH ratio released by the gland. Thus, with I deficiency, a larger proportion of the circulating T3 may be produced thyroidally than with adequate I availability (see review by Taurog, 1986). In contrast, when I is not limiting, T3/T4 ratios in the blood are lower and up to 80% of the circulating T3 is produced peripherally (see review by Kaplan, 1986).

The TG has some autoregulatory ability to adjust to I deficiency. Decreased thyroidal I enhances the sensitivity of the gland to TSH stimulation. TSH stimulation of an I deficient gland causes I uptake greater than that obtained with either condition separately (see review by Studer et al., 1974).

I shortage also can cause alterations in TG structure (rats: review by Taurog, 1986; man: review by Erman, 1986). Increased mass of the TG due to hyperplasia and hypertrophy of the cells is found because of the increase in TSH stimulation. This is an adaptive response to I deficiency in which the increased growth leads to additional functional capacity of the gland. Prolonged TSH stimulation is the result of the failure of the gland to compensate for the I shortage. However, extreme enlargement of the gland (i.e. goiter) can actually reduce the efficiency of the TG to synthesize TH. Beyond the point at which the gland can trap 100% of the available I, Tg iodination can be impaired due to reduced I/Tg ratios (see reviews by Studer et al., 1974; Ermans, 1986).

Thus, there is a general pattern by which the TG adjusts to I deficiency in mammals. Only under extreme and prolonged conditions of I shortage do the regulatory mechanisms fail to compensate in the maintenance of circulating TH concentrations.

B. Birds

1. Thyroid Function

Reduced dietary I repeatedly has been found to result in decreased I content of the TG of chickens (Creek et al., 1957; Rogler et al., 1959b, 1961a; Rosenberg et al., 1963; Newcomer, 1978). TG-I is directly proportional to dietary I below some basal level of I intake (150-300 μg/kg) (chicken: Creek et al., 1957; Newcomer, 1978; quail: McNabb et al., 1985a). Reduced TG-I is also seen in embryos from hens fed low I diets (chicken: Rogler et al., 1959a, 1961b; quail: McNabb et

al., 1985b). However, the TG of hens has been found to be very resistant to TG-I depletion (Rogler et al., 1961a).

The proportion of I present in the TG as iodotyrosines is altered with reduced TG-I. Decreased TG-I appears to favor formation of MIT over DIT resulting in reduced T4 synthesis as has been found in mammals. The TG of chickens fed a low I diet (140 μg/kg) contained an increased MIT/DIT ratio (Rosenberg et al., 1963). This change in MIT/DIT ratios is reflected in increased T3/T4 ratios such as those found in quail fed a diet low in I (McNabb et al., 1985a).

Increased TG weight and altered TG histology often have been used as an indicator of TSH stimulation. Reduced circulating TH concentrations are detected by the pituitary which releases TSH, stimulating TG growth, increased cell number and size. Many studies of birds have reported signs of TSH stimulation with I deficiency. Reduced I availability results in elevated TG weight (Scott et al., 1960; McNabb et al., 1985b) and increased cell height, hyperplasia and reduced amounts of colloid (Creek et al., 1957; Rogler et al., 1959b, 1962). With low but not severe I deficiency (< 100 μg/kg), TG weight may not increase but TG histology may exhibit signs of TSH stimulation (Creek et al., 1957; Rogler et al., 1961a&b; Newcomer, 1978). However, some researchers have found no increases in TG weight with low dietary I (< 50 μg/kg)(Newcomer, 1978; McNabb et al., 1985a).

TSH stimulation causes increased I uptake by the TG due to production/synthesis of proteins for active transport units. Low I availability can result in TSH stimulation, reflected in increased radioiodine uptake by the TG. Below basal dietary I levels (150-300 μg/kg), the percent uptake of radioiodine has been found to be inversely related to I in the diet (chickens: Rogler et al., 1961a&b; Rosenberg et al., 1963; quail: McNabb et al., 1985a) or I in the egg (chickens: Rogler et al., 1959a&b, 1961b, 1962; Featherston, 1966; quail: McNabb et al., 1985b). In addition to increased uptake of labelled I, the absolute amount of I, i.e. labelled plus unlabelled, trapped by the TG following I administration is increased with reduced I availability (Newcomer, 1969). Radioiodine clearance (volume of serum required to account for the radioiodine in the gland) and the thyroid I/serum I ratio also reflect the gland's ability to concentrate I. Both parameters have been found to be elevated with I deficiency (Newcomer, 1968, 1969, 1978; Davison et al., 1981).

The TG of I deficient birds takes up a larger percent of radioiodine more rapidly than the TG of birds fed diets adequate in I. However, the percent of radioiodine dose remaining in the gland was found to decline more rapidly with reduced I availability (Rogler et al., 1961b, 1962) and appear more quickly in the periphery, compared to birds who had adequate I availability (Rogler et al., 1962; Featherston et al., 1966). This implies a more rapid utilization or turnover of radioiodine within the gland under low I conditions.

Following I administration, the fraction of I incorporated into protein within the gland was larger when I availability was limited than with adequate I available (Newcomer, 1968, 1978). The increased protein bound I within the gland, which includes hormonal-I, is proportional to the increased amounts of I taken up by the gland (Newcomer, 1969). TSH stimulation causes the TG to compensate for reduced I availability by increasing the amount of I taken up by the gland and, evidently, rapidly utilizing the I in TH formation for release to the circulation.

Circulating TH concentrations usually are not altered with low I. A tendency toward reduced T4 concentrations has been found with very reduced I availability (chickens: Newcomer, 1978), whereas moderately low I had no effect on serum TH concentrations (quail hens and embryos: McNabb et al., 1985a&b). Reduced circulating protein bound I (PBI) concentrations have been reported with I deficiency by some investigators (Rosenberg et al., 1963; Astier, 1975), but not others (Singh et al., 1968). However, with the availability of sensitive radioimmunoassays, PBI has been found not to be an accurate estimate of TH concentrations (Astier, 1975). In general, it appears that circulating TH concentrations are maintained despite limited I availability. With severe I deficiency, total TH levels may be reduced but the alterations in function are such that concentrations of the metabolically active hormone, T3, are maintained.

2. Embryonic and Posthatching Growth

Because I is an essential component of TH and TH are necessary for normal growth, many studies of I deficiency in avian species have attempted to determine the level of dietary I needed to support normal growth. Several avian species appear to be fairly resistant to the adverse effects of low I availability on posthatching growth. No reductions in body weight were found with lowered dietary I (pheasant and quail: Scott et al., 1960; chicken: Rogler et al., 1961a, Newcomer, 1978; quail: McNabb et al., 1985a). Only with extremely low I (< 35 μg/kg feed) is growth reduced in growing chickens (Creek et al., 1957).

The I content of a hen's diet determines the I content of the eggs produced (Wilder et al., 1933). Developing oocytes actively trap I for deposition in the egg yolk (Newcomer, 1984). However, when I is limited, thyroidal I uptake may be favored because the TG is more active at concentrating I than are the ovaries (Robinson et al., 1977), and because I uptake by the TG is regulated by TSH (Newcomer, 1982). As a result, egg yolk I is inversely related to maternal dietary I (Rogler et al., 1959a; McNabb et al., 1985a).

Using this relationship between maternal dietary I and egg I, some studies have addressed the effect of reduced maternal dietary I on egg production and embryonic growth. Reduced maternal dietary I caused lowered hatchability, and up to five day increases in incubation time (Rogler et al., 1959a). These effects appear to be due to reduced I availability to the TG of the embryo. Egg production, weight and fertility are not altered with low maternal I (Rogler et al., 1959a, 1961b). Introduction of I to the chorioallantoic membrane of low I eggs significantly increased the hatchability (Rogler et al., 1959a). Growth in embryos, like young birds, can be adversely affected with severe I deficiency (< 30 μg/kg feed maternal diet, Rogler et al., 1961b). Reduced body weight has been found in chicken embryos from low I eggs, as early as day 16 of the 21-22 day incubation; the magnitude of the growth reduction increased to day 20 (Rogler et al., 1959a). With moderately low maternal dietary I (50 μg/kg) body weight of embryos and hatchlings was unaffected (quail: McNabb et al., 1985b).

V. The Effects of High Iodine Availability

A. Mammals

The TG has a variety of mechanisms for adjusting to fluctuations in I availability. Although TSH is the major regulator of TG function, autoregulatory mechanisms predominate in compensatory action when I availability is in excess of I required for TH formation.

1. Acute Effects

The initial effects of increased I availability are stimulatory to TG function. Increased circulating I concentrations first result in increased I transport into the TG cell. I is typically the rate limiting factor in the iodination of Tg. Elevated intracellular I causes increased formation of organic-I compounds (see reviews by DeGroot et al., 1984; Taurog, 1986; Utiger, 1987). There also may be a slight elevation in TH release from the gland (see reviews by DeGroot et al., 1984; Martin, 1985). However, these stimulatory effects of high I are transient.

Continued elevation of I availability results in an inhibition of Tg iodination, i.e. decreased percent incorporation of I and decreased rate of iodination, which is termed the Wolff-Chaikoff effect (see reviews by Bastomsky, 1974; Nagataki, 1974; DeGroot et al., 1984; Nagataki and Ingbar, 1986). For a brief period, I continues to be transported into the gland, but not incorporated into Tg, resulting in an increased intracellular I concentration.

This I-induced inhibition of iodoamino acid formation appears to be caused by some unidentified iodinated organic compound. Methimazole and thiouracil, which block iodination reactions, prevent the Wolff-Chaikoff effect (see reviews by Nagataki, 1974; Martin, 1985; Nagataki and Ingbar, 1986; Utiger, 1987). The mechanism by which the inhibitor reduces I incorporation into

Tg is unknown. Lowered production of H_2O_2 has been implicated in the Wolff-Chaikoff effect (see reviews by DeGroot et al., 1984; Martin, 1985; Taurog, 1986). Concentrations of pyridine nucleotides, which are required for H_2O_2 generation, have been found to be reduced with high I availability (Maayan and Ingbar, 1970). Alternatively, formation of I_3^-, an inefficient iodinator, also has been suggested as the mechanism of Wolff-Chaikoff when I concentrations are increased (see reviews by DeGroot et al., 1984; Nagataki and Ingbar, 1986).

In addition to the quantitative changes in TH synthesis, i.e. a reduction in Tg iodination, the relative proportions of iodoamino acids formed are altered during the I-induced inhibition. The percentage of MIT residues present increases during the Wolff-Chaikoff effect. Coupling is reduced as shown by the relative decrease in T3 and T4 formed (see reviews by Nagataki, 1974; DeGroot et al., 1984; Nagataki and Ingbar, 1986). TH release also is reduced during the acute inhibition of iodination of Tg (see reviews by DeGroot et al., 1984; Norman and Litwack, 1987). Perhaps this is due to the relatively low amounts of TH contained in the Tg iodinated during the Wolff-Chaikoff inhibition.

The inhibition of Tg iodination is transient and disappears in approximately forty-eight hours, despite continued elevation of circulating I concentrations. This "escape" from the Wolff-Chaikoff effect is due to a reduction in I transport activity. Lowered intracellular I concentrations become insufficient to maintain the inhibitory effects of I on organification (see reviews by Nagataki, 1974; DeGroot et al., 1984; Nagataki and Ingbar, 1986; Taurog, 1986; Norman and Litwack, 1987; Utiger, 1987).

The mechanism by which I transport is decreased is unknown but results in an increased Km of the transport for I (see review by Norman and Litwack, 1987) and may be caused by lowered ATP (Maayan and Ingbar, 1970) and cAMP concentrations in the gland associated with elevated intracellular I (see reviews by Nagataki and Ingbar, 1986; Norman and Litwack, 1987; Utiger, 1987). Following the release of inhibition, iodination of Tg resumes without the alterations in iodoamino acid synthesis previously mentioned.

Sensitivity to the inhibitory effects of increased plasma I concentrations is determined by the functional state of the gland. Glands in which I transport is highly active require less I adminis-

tration to cause the inhibition. Rats pretreated with TSH exhibit the Wolff-Chaikoff effect at much lower I concentrations than those not stimulated (see review by Nagataki and Ingbar, 1986). Similarly, rats fed a diet deficient in I show inhibitory effects of increased I at one-tenth the level of I required to show the effect in rats fed diets adequate in I (see review by Taurog, 1986). Evidently, any stimulation of the gland that causes increased I transport activity raises intracellular I concentrations more rapidly, initiating the autoregulatory mechanisms.

2. Chronic Effects

After chronic exposure to elevated circulating I concentrations, that is, following the escape from the acute inhibitory effects of increased I, the autoregulatory mechanisms of the TG compensate for the sustained elevation in I availability. Adaptation includes a reduction in I transport activity. However, due to the increase in peripheral I available to be trapped, the quantity of I accumulated and organified is actually elevated compared to lower I availabilities (see reviews by Nagataki, 1974; Nagataki and Ingbar, 1986, Taurog, 1986). Despite the elevation in I taken up and utilized in Tg iodination, Tg turnover, secretion of TH, and circulating TH concentrations are not altered (see reviews by Nagataki, 1974; Taurog, 1986). Several factors contribute to the lack of increased TH release despite increased Tg iodination. As Tg is progressively iodinated in vitro, iodothyronine content plateaus while iodotyrosine residues, particularly DIT, continue to increase (see reviews by Studer et al., 1974; Taurog, 1986). Thus, hydrolysis of highly iodinated Tg , as is found when I availability is elevated, does not necessarily result in increased amounts of TH being released from Tg and the TG.

Typically, the deiodination of iodotyrosines released following proteolytic degradation of Tg provides the TG with a secondary source of I for reuse in the iodination of Tg. With adaptation to chronic elevation of I, this second pool of I is not efficiently utilized and leaves the gland as non-hormonal I (see reviews by Nagataki, 1974; DeGroot et al., 1984; Nagataki and Ingbar, 1986;

Taurog, 1986). This autoregulatory mechanism is often called an "iodine leak". Evidently, I trapped by the gland is organified preferentially, and intracellularly generated I is released as a mechanism to maintain the I balance within the gland when I availability is increased.

In addition to the autoregulatory effects of high I within the TG, there is a modulation of TSH effects on the gland following sustained exposure to elevated I concentrations. Chronically increased I reduces follicular cell sensitivity to TSH (see reviews by Martin, 1985; DeGroot et al., 1984; Nagataki and Ingbar, 1986). Binding of TSH to basal membrane receptors is not altered, yet TSH-induced release of TH is reduced. This inhibition of TH release by I may be due to a reduction in cAMP production which is responsible for most, if not all, of the stimulatory effects of TG function by TSH (see reviews by Martin, 1985; Nagataki and Ingbar, 1986; Norman and Litwack, 1987; Utiger, 1987).

Inhibition of Tg proteolysis also may be involved in the reduction of TH release found after chronic exposure to increased I (see reviews by DeGroot et al., 1974; Martin, 1985; Utiger, 1987). Some studies report decreased Tg degradation when TG-I is elevated (Takeuchi et al., 1970; Santisteban and Lamas, 1981; see review by Nagataki and Ingbar, 1986).

Both man and rat, which have been studied most intensively, have excellent ability to adjust TG function despite high I through autoregulatory mechanisms and their interaction with TSH effects. Other mammalian species have recieved limited study, but there appear to be differences between species in the ability of the TG to compensate for increased I availability. In contrast to rats, mice are very susceptible to I-induced increases in TG weight. In mice, excess I, both acutely and chronically, supresses TH release from the gland, yet Tg iodination is greatly increased (Itikawa et al., 1967a&b). Lowered circulating TH concentrations caused increased TSH stimulation of the TG resulting in elevated TG weight. Myxedema, and hypertrophy of the gland due to excess I, have been reported to occur in man and appear to be linked to a failure to escape from the acute inhibitory effects of high I (review by Wolff, 1969). Guinea pigs treated with excess I show histological evidence of TG stimulation (see review by Nagataki and Ingbar, 1986).

B. Birds

The mechanisms by which the TG adjusts to increased I availability have received less attention in birds than in mammals. However, it appears that the TG of birds also has autoregulatory mechanisms which compensate for elevated circulating I concentrations.

1. Acute Effects

A transient reduction in organic binding of I several hours after I administration has been reported in chickens (Suzuki et al., 1969). The reduced organification of I was accompanied by an elevated MIT/DIT ratio and lowered T4 synthesis within the TG. This response is characteristic of the Wolff-Chaikoff effect found in mammals (see previous dicussion). Although other research (Newcomer, 1969) reported no inhibition of organic-I formation in chickens fed a diet adequate in I, the response was determined only at one time period (one hour following I administration). This may have been too early for the inhibitory effect of excess I to be initiated. In addition, the same study reported a reduction in I organification in the TG of birds fed a diet relatively low in I. Stimulated glands in mammals, as occurs with I deficiency, are more sensitive to the inhibitory effect of elevated I. Thus, from the limited information available, it appears that the TG of birds responds to high I by a temporary reduction in organic binding of I, much like the Wolff-Chaikoff effect seen in mammals.

Research with mammals has shown that the Wolff-Chaikoff effect is followed by reduced I transport from the circulation into the follicular cell. This autoregulatory mechanism has not been adequately studied in birds. One study showed increased amounts of I within the gland with increasing I administration (Mayberry and Hockert, 1968). This was interpreted as indicating no diminution in I transport with excess I. However, when uptake is expressed as a percent of I available, a reduction in I transport is found. More research is needed to determine if I transport

is inhibited when I concentrations are elevated as an autoregulatory mechanism to prevent excessive I entering the gland and/or to escape from the inhibition of Tg iodination.

2. Chronic Effects

Sustained exposure to elevated peripheral radioiodine availabilities results in reduced I transport into the TG of birds (Newcomer, 1978). Despite reduced uptake, I content of the gland is increased in some studies (Suzuki, 1969; Takeuchi, 1970; Newcomer, 1978; quail embryos: McNabb et al., 1985b) but not in another (quail hens: McNabb et al., 1985a).

T4 content of the gland has been reported to be elevated with high I availability (Suzuki, 1969). However, TG-T4 was reduced when expressed as a percent of I within the gland. It appears that increased thyroidal I did not necessarily result in increased TH formation within Tg. Reduced MIT/DIT ratios following elevated I availability also were found in this study as have been found in mammals. Tg hydrolysis was decreased in chicks chronically exposed to excess I; the decrease is due to reduced proteolytic activity, not changes in the resistance of Tg to degradation (Takeuchi et al., 1970).

With excess I availability, circulating TH concentrations are not altered (chickens: Mayberry and Hockert, 1968; Suzuki et al., 1969; Newcomer, 1978; quail hens and embryos: McNabb et al., 1985a&b). Peripheral turnover of TH (a measure of TH degradation/excretion) also was not different (Suzuki et al., 1969). Although little is known concerning the autoregulatory mechanisms of the TG in birds, the gland maintains circulating TH concentrations despite increased I availability.

Despite the ability of the TG to maintain peripheral TH levels, some studies have reported increased TG weight following exposure to excess I (Wheeler and Hoffman, 1949; Suzuki et al., 1969; Sawada et al., 1967 and Takahashi et al., 1967 as cited by Newcomer, 1978; Takeuchi et al., 1970). Elevated TG weight could be due to increased TSH stimulation following reduced TH concentrations. However, other researchers have found no difference in TG weight with high I availability

(Mayberry and Hockert, 1968; Newcomer, 1978; McNabb et al., 1985a&b). The reason for these differences among studies is unknown but may relate to differences in age or strain of animals, level of I supplementation or route of I administration.

Developing oocytes are known to accumulate I (Robinson et al., 1977) and egg yolk I content is directly related to maternal dietary I (McNabb et al., 1985a). The effect of excess I on egg production and embryo development also has been studied. Increased maternal dietary I has no effect on production or fertility of eggs (McNabb et al., 1985a) except with very high I intake (Perdomo, 1966). But, elevated yolk I has been reported to cause increased incubation time and mortality of the embryos (Wheeler and Hoffman, 1949; Perdomo, 1966) and reduced body weight in young animals in some studies (Mayberry and Hockert, 1968) but not others (Newcomer, 1978; McNabb et al., 1985b)

More research is needed to further elucidate the regulatory mechanisms by which the TG of birds adapts to sustained exposure to elevated I. It appears that compensation occurs to achieve the maintenance of circulating TH concentrations. Whether the autoregulatory mechanisms of birds are similar to those of mammals remains unclear.

VI. Control of Thyroid Gland Function

A. Mammals

Although autoregulatory mechanisms operate within the gland, TSH from the anterior pituitary is the major regulator of TG function (see reviews by Tong, 1974; Hadley, 1984; Field, 1986; Norman and Litwack, 1987). Binding of TSH to membrane receptors on the basal surface of the follicle cell stimulates virtually all aspects of TG function. Augmented colloid endocytosis and Tg

proteolysis is found within minutes after TSH binding, resulting in increased TH release. TSH also stimulates TH synthesis through increased incorporation of I into Tg. Several hours after TSH-receptor interactions, Tg synthesis is increased and I uptake is elevated. TSH also stimulates many aspects of carbohydrate, phospholipid and protein metabolism. Continued TSH stimulation causes structural changes in the cell related to increased production of biosynthetic apparatus such as rER and golgi. These changes can result in hypertrophy of the cell. Chronically stimulated glands may exhibit hyperplasia due to increased mitosis.

TSH effects and autoregulatory mechanisms both influence TG function. There is a modulation of TSH effects on the gland when I induced autoregulatory effects are operating. Follicular cell sensitivity to TSH is reduced following chronic exposure to elevated I availability (see reviews by DeGroot et al., 1984; Martin, 1985; Nagataki and Ingbar, 1986). Reduced I availability causes enhanced responses to TSH stimulation (see review by Studer et al., 1986).

Most of the effects of TSH are mediated through cAMP, the most studied of the second messenger systems in the TG (see reviews by Hadley, 1984; Martin, 1985; Taurog, 1986). However, other mechanisms of action due to TSH binding are known to exist within the gland. Increased intracellular calcium, causing calcium-calmodulin binding, and increased phospholipid metabolism, resulting in elevated inositol triphosphate and diacylglycerol occur following TSH stimulation. In addition, glucose transport is increased, and this may be mediated through the other systems. Each of these mechanisms is known to trigger physiological effects through the activation of specific protein kinases. However, the full biochemical pathways and relative importance of these second messenger systems has not been clearly elucidated (see reviews by Norman and Litwack, 1987; Tepperman and Tepperman, 1987).

TSH is produced and released from thyrotropic cells of the anterior pituitary. Negative feedback effects of TH directly on the thyrotrophs constitute the predominate factor influencing TSH secretion. Secretion of TSH also is influenced by the hypothalamus through the stimulatory effects of TRH and the inhibitory effects of somatostatin. Thus, through an interaction between the various stimulatory and inhibitory influences on the anterior pituitary, circulating TH are maintained

within a narrow range of concentrations (see reviews by Norman and Litwack, 1987; Utiger, 1987; Tepperman and Tepperman, 1987).

B. Birds

Although less information is avilable in birds and avian TSH has not been purified, TSH appears to exert its control of TG function in birds in the same manner as occurs in mammals. Increased numbers of colloid droplets are found in the cell following TSH stimulation (see reviews by Falconer, 1971; Wentworth and Ringer, 1986). Elevation of plasma TH concentrations has been demonstrated in several avian species after TSH stimulation (see review by Scanes, 1986). TSH also stimulates production of TH, as indicated by increased TG-T4 content, radioactive phosphate and I uptake (McNabb et al., 1986). This study demonstrated that chronic TSH stimulation (at least 3 days) was needed to elevate 125-I uptake in Japanese quail. Thus, birds may require a longer exposure to TSH to increase I uptake than has been found for mammals. Increased TG size is found following exogenous TSH treatment (Robinson et al., 1976) and elevated endogenous TSH concentrations due to low I availability (see previous discussion). cAMP is implicated as the second messenger for most of these effects. TG stimulation by TSH causes elevated cAMP concentrations *in vitro* (Tonoue and Kitoh, 1978) and *in vivo* (McNichols and McNabb, 1988).

TSH production and release in birds appears to be under the same regulatory influences as have been demonstrated in mammals. TRH stimulates TSH release from the pituitary; plasma TH concentrations and TG uptake of phosphate are increased presumably through TRH-induced release of TSH (see review by Wentworth and Ringer, 1986). Negative feedback effects of TH on pituitary release of TSH have been demonstrated (see review by Falconer, 1971; Wentworth and Ringer, 1986) although TH inhibition of hypothalamic stimulation may be involved also (see review by Scanes, 1986). Thus, thyroid function appears to be regulated by hypothalamic-pituitary mechanisms in much the same manner in avian species as in mammals.

Introduction

The I content of a hen's diet directly affects the egg I content in galliform birds (chickens: Wilder et al., 1933; Rogler et al., 1959a; quail: McNabb et al., 1985a). Over a wide range of I availabilities, maternal dietary I does not alter egg weight, production, or hatchability (Rogler et al., 1961a&b; McNabb et al., 1985a) except with extremely low I intakes (Rogler et al., 1959a, 1961b) or extremely high I intakes (Perdomo et al., 1966). Egg I is taken up and utilized by the TG of the developing embryo and the TG-I content of embryos and chicks is directly related to egg I (chickens: Rogler et al., 1959a, 1961b; quail: McNabb et al., 1985b). With low egg I, and thus low TG-I, embryonic and hatchling TG weight is elevated and radioiodine uptake is increased (chickens: Rogler et al., 1959b, 1961b, 1962; quail: McNabb 1985b) which suggest a pituitary response to reduced plasma TH concentrations during development. With increased TG-I, TG weight usually is not altered (Mayberry and Hockert, 1968; Newcomer, 1968, 1978) although there are reports of elevated TG weight with increased I availability (chickens: Wheeler and Hoffman, 1949; quail: McNabb et al., 1985b).

Although only limited information is available, plasma TH concentrations are maintained despite altered TG-I in embryos (McNabb et al., 1985b) and chicks (Newcomer, 1978; McNabb et al., 1985b). Over a broad range, I availability is not associated with differences in body weight in

embryos (Creek et al., 1957; Rogler et al., 1961b) or chicks (Scott et al., 1960; Newcomer, 1978). This provides circumstantial evidence that circulating TH concentrations are not reduced because adequate TH are needed for normal growth in birds (review by King and May, 1984). However, reduced body weight is reported with extremely low I availability (Creek et al., 1957; Rogler et al., 1959a, 1961b) and extremely high I availability (Mayberry and Hockert, 1968). Generally, it appears that circulating TH are maintained despite a wide range of I availabilities.

Although peripheral TH do not differ with different I availabilities during development, no studies have addressed the thyroid dynamics that achieve the maintenance of plasma TH concentrations. To investigate these dynamics, Japanese quail with different I availabilities were used to study thyroid function during development. The following two developmental stages were investigated: (1) embryos on day 14 of a 16.5-17 day incubation period, a time of increasing capacity for TG function but relatively low requirements for TH, (2) chicks at 24 hours after hatching, a time at which there are increased demands on the TG but before dietary I influences. To provide more extensive information, the effect of different I availabilities on the developmental patterns of TH concentrations in the TG and plasma were described. In the present study, low I availability ($\leq$50 μgI/kg feed in the maternal diet) and high I availability (1200 μgI/kg feed) were compared to control levels (800 μgI/kg feed), a common I supplementation for gamebirds.

Methods

Animals

Fertile eggs were obtained from the Poultry Science Department at VPI&SU from random-bred colonies of Japanese quail (*Coturnix japonica*). Hens were fed one of three dietary I treatments: (1) a purified dextrose soy-protein based diet containing $\leq$50 μgI/kg feed (analysis by Hazelton Laboratories Inc., Madison, WI), (2) commercial game bird feed (Big Spring Mills, Elliston, VA) supplemented to 800 μgI/kg, or (3) commercial game bird feed supplemented to 1200 μgI/kg. Eggs were incubated at 39 $\pm$ 1 C and greater than 90% relative humidity in a forced air incubator. Chicks were maintained at 37 $\pm$ 1 C on a photoperiod of L:D 14:10, and provided with water but no food for the first 24 hours.

Thyroid Gland Iodine Analysis

Thyroid glands were digested in perchloric acid and I content was determined using a spectrophotometric assay utilizing the decolorization of ceric to cerous ions in the presence of arsenious acid and I (based on a commercial method by Hycel Inc., Houston, TX as modified by McNabb et al., 1985a).

Plasma Thyroid Hormones Concentrations

Blood was collected into heparinized microhematocrit tubes from the chorioallantoic arteries of embryos or the jugular veins of chicks killed by decapitation. After samples were centrifuged, plasma was separated and stored frozen at -10 C until analysis.

Plasma T3 and T4 concentrations were determined using a double antibody RIA verified for use with quail plasma (McNabb and Hughes, 1983). A T4 precision test indicated that ± 2SE was 2.6% of the mean (n = 10) and accuracy was 97.1% against a euthyroid control serum (Lyphochek Immunoassay Control Level I, Biorad Inc., Anaheim, CA). For T3, a precision test indicated that ± 2SE was 4.7% of the mean (n = 10) and accuracy was 100.5% against the same control serum.

Thyroid Gland Hormone Content

To determine the hormone content of the TG (method of McNabb and Cheng, 1985), glands were homogenized in 100μl of Tris buffer and digested for 24 hours with 5X the thyroid weight of Pronase (Calbiochem-Behring, San Diego, CA) and added buffer for a final volume of 500μl. Hormones were extracted in 95% ethanol and the ethanol extract was stored at -10 C until analysis by a double antibody RIA using ethanol-based standards.

Thyroid Gland Response to TSH

Bovine TSH (Sigma Chemical Co., St. Louis, MO) was used to assess the responsiveness of the TG of 1 day chicks from the different I groups. TSH (25mU in 20 μl of 0.9% NaCl) or the equivalent amount of saline, was injected subcutaneously into the nape of the neck. This dose was chosen as the minimum amount of TSH to cause the maximum physiological response in chicks (McNabb et al., 1986). The response of the TG to TSH was evaluated by two types of experiments:

1. The cAMP content of the gland 30 minutes after TSH injection was determined. After chicks were decapitated, the TG was removed quickly and frozen immediately on dry ice. The TG were homogenized in 100μl of 0.1 N HCl with 0.2mM isobutylmethylxanthine (a phosphodiesterase inhibitor, Sigma Chemical Co., St. Louis, MO). After centrifuging the homogenate for 5 minutes at 11,000g, the supernatant was frozen at -10 C until analysis by a cAMP RIA (McNichols and McNabb, 1988) in which both samples and standards were succinylated to increase the sensitivity of the assay. A precision test of the cAMP RIA indicated that $\pm$ 2SE was 3.2% of the mean (n = 10).

2. TG release of TH in response to TSH stimulation was evaluated by determining plasma T3 and T4 concentrations from 1/2 to 8 hours after injection. Blood was collected and plasma TH concentrations were determined as described above.

Hepatic 5'Monodeiodinase Activity

The enzymatic activity of 5'-D was determined by the method of Hughes and McNabb (1986). Livers were removed, weighed and homogenized in 3 volumes of buffer [0.05M MOPS (morpholinopropane sulfonic acid), 1.0mM EDTA, pH 7.4]. Specific details of the method (including modifications developed by Freeman, unpublished, our laboratory) were: the homogenate was diluted in MOPS buffer to provide a 5% (w/v) solution and a 50 μl aliquot was incubated for 15 minutes at 37 C with substrate (4.0μM T4), cofactor (1.0mM dithiothreitol) and MOPS buffer in a total volume of 80 μl. After the incubation, the reaction was stopped by the addition of 75 μl EtOH. The T3 was extracted for 24 hours at 0 C, then centrifuged at 4000g for 20 minutes at -10 C. The ethanol extract was stored at -10 C until analysis for T3 by double antibody RIA using ethanol based standards. Enzyme activity is expressed as ng T3/mg tissue-minute.

Body Weight and Thyroid Gland Weight

Body weight was recorded (to 0.01 g) at the time of sampling. Thyroid gland weight (Wt) was recorded (to 0.01 mg) immediately after decapitation and removal of the gland at the time of sampling.

Experimental Design and Statistical Analysis

Experiments were performed on 14 day embryos and 1 day old chicks, except in experiments to determine the TG response to TSH in which only 1 day chicks were used. In addition, to describe developmental patterns, TG-TH and plasma TH were measured on embryonic days 10, 12, 14, 16, on the day of hatching, and 1 day posthatching. During the perinatal period, the stage of hatching

was recorded i.e. pipped through the chorioallantoic membrane or pipped through the shell. Plasma hormone concentrations were compared among the I groups for animals in the same stage of hatching.

The results of these studies were analyzed statistically using Analysis of Variance with Duncan's Multiple Range Test to indicate treatment differences. A value of $p \leq .05$ was accepted as indicative of statistically significant differences.

Results

Thyroid Gland Iodine Content

TG-I content of embryos and chicks was related positively to maternal dietary I. Day 14 embryos and 1 day chicks from low I eggs had significantly reduced total TG-I and weight-specific TG-I compared to controls (Figure 1). Embryos and chicks with high I availability showed significantly increased total TG-I and weight-specific TG-I (Figure 1).

Thyroid Gland Hormone Content

With low I availability, TG-T4 content was significantly reduced compared to control values in both embryos and chicks (Figure 2). TG-T4 was not different between the high I group and controls in 14 day embryos or 1 day chicks (Figure 2). Thyroid gland T3 content was not different among different I groups in either embryos or chicks (Figure 2).

TG-TH also were compared across a sequence of ages. With low I availability, TG-T4 was significantly reduced compared to controls on day 14 and thereafter (Figure 3). However, the T4 content of the gland increased in parallel to the control group with peak values of TG-T4 occuring in 1 day chicks. No significant differences were found in TG-T4 with high I availability compared to controls (Figure 3).

The developmental pattern of TG-T3 content was not different with low or high I availability compared to controls (Figure 4). The results for TG-T4 and TG-T3 were comparable when these data were expressed as weight-specific hormone content (data not shown).

Plasma Thyroid Hormone Concentrations

Plasma T4 and T3 concentrations were not different between the I groups in either 14 day embryos or 1 day chicks (Figure 5).

When the developmental patterns of plasma TH were determined, no differences were found in the patterns of plasma T4 (Figure 6) or T3 (Figure 7) among the I availability groups. Plasma T4 concentrations rose from day 10 until day 16 and peak values preceeded those for T3. Plasma T3 concentrations remained low until the perinatal period (day 16), then peaked as the chicks pipped through the shell. Plasma TH were not significantly different among the I groups at any age, but the mean values for T3 and T4 tended to be reduced in the high I group compared to controls for most ages sampled.

Thyroid Gland Response to TSH

1. TG-cAMP content - cAMP content of the TG of 1 day chicks was significantly elevated following TSH injection compared to saline injection (Figure 8). The effect was the same regardless of I availability group.

2. Plasma TH - TSH-injected chicks had significantly elevated plasma T4 concentrations compared to saline-injected chicks at each time period sampled. However, the magnitude of the plasma T4 response to TSH did not differ between high I and control chicks (Figure 9) or low I and control chicks (Figure 10).

Plasma T3 concentrations did not change in response to TSH in any I group at any time period sampled (data not shown).

Hepatic 5′-D Activity

There were no differences in hepatic 5′-D activity among the I groups in either 14 day embryos or 1 day chicks (Figure 11).

Body Weight and Thyroid Gland Weight

Low I availability had no significant effect on body weight in embryos or chicks. Body weight was significantly reduced in embryos and chicks with high I (Table 1).

No differences were found in Wt among the I groups in 14 day embryos or 1 day chicks (Table 2).

High Iodine Individuals with Hypertrophied Thyroid Glands

A few individual embryos and chicks (9%) of the high I group exhibited marked hypertrophy of the TG (3X mean weight). When data for these individuals were pooled, several parameters are found to be different from the rest of the high I group. Individuals with hypertrophied TG showed significantly increased TG-T3 and TG-T4 content and significantly decreased plasma T3 and T4 concentrations (Table 3). Weight specific TG-T3 and TG-T4, and body weight were not different between the individuals and the other high I embryos and chicks (data not shown).

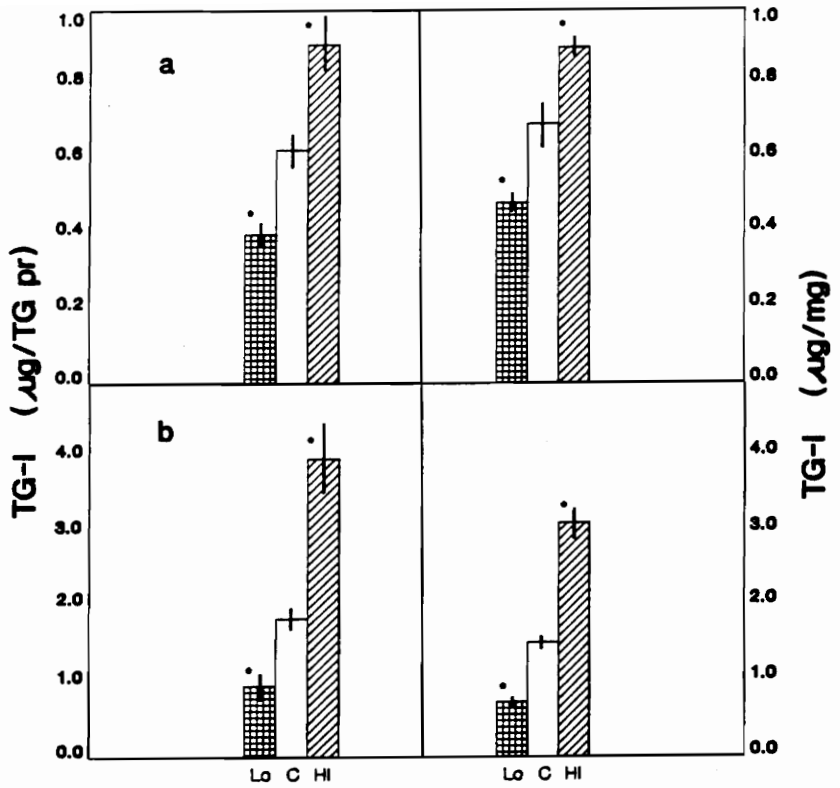

Figure 1. Thyroid gland I content of 14 day embryos (a) and 1 day chicks (b) of Japanese quail from eggs of hens fed diets with different I availabilities. Low I (Lo): ≤50 µg/kg feed, control I (C): 800 µg/kg feed, high I (Hi): 1200 µg/kg feed. Values are means ± 2SE (n = 8-10). Asterisks designate significant differences from control values ($p \leq .05$).

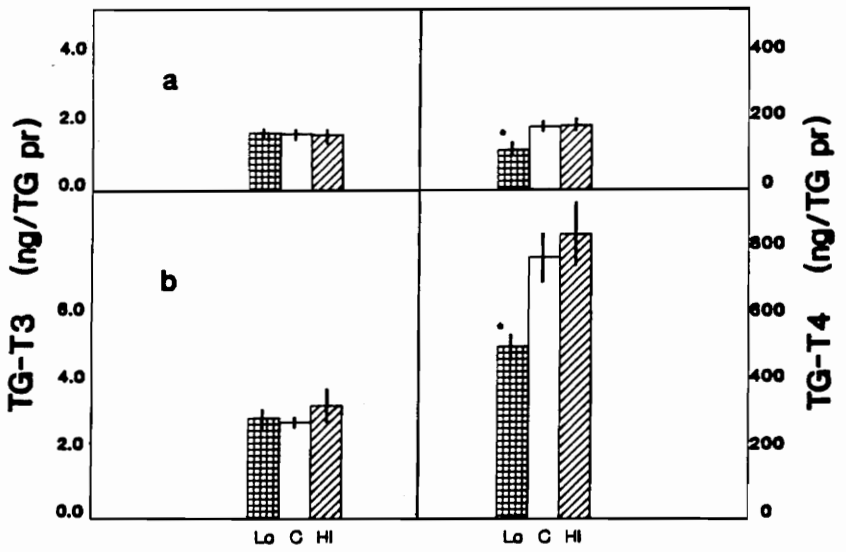

Figure 2. Thyroid gland hormone content of 14 day embryos (a) and 1 day chicks (b) of Japanese quail from eggs of hens fed diets with different I availabilities. Low I (Lo): ≤50 μg/kg feed, control I (C): 800 μg/kg feed, high I (Hi): 1200 μg/kg feed. Thyroid gland T3 content: TG-T3, thyroid gland T4 content: TG-T4. Values are means ± 2SE (n = 8-10). Asterisks designate significant differences from control values ($p \leq .05$).

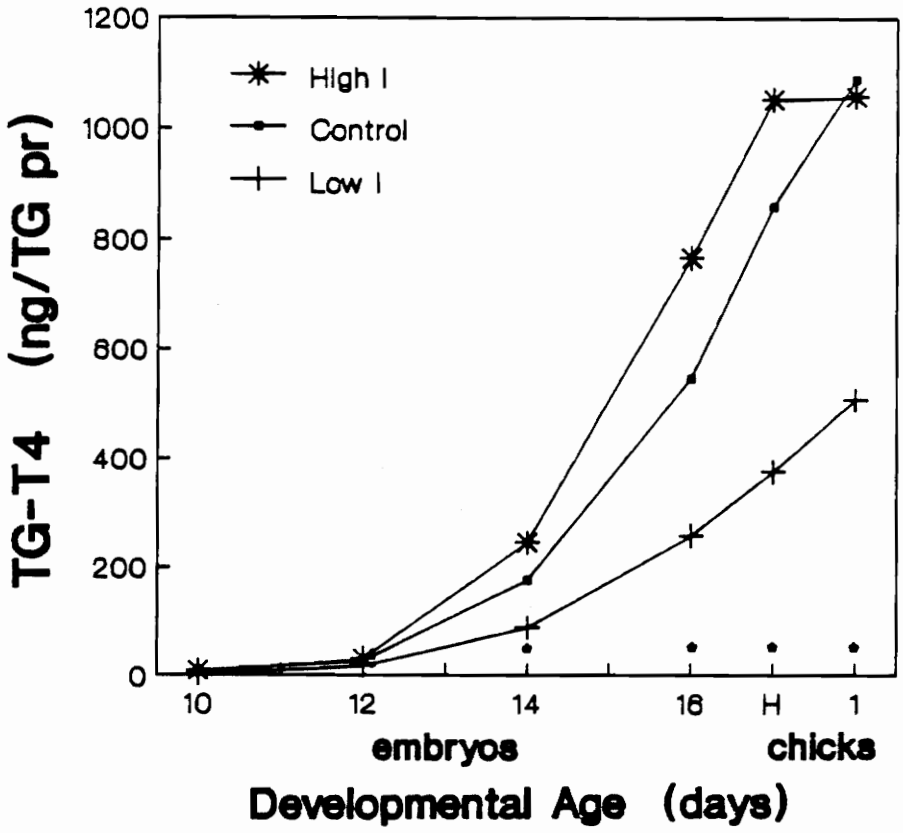

Figure 3. Developmental pattern of thyroid gland T4 content of Japanese quail embryos and chicks from eggs of hens fed diets with different I availabilities. Low I: ≤50 μg/kg feed, control I: 800 μg/kg feed, high I: 1200 μg/kg feed). H designates chicks on the day of hatch. Values are means at each age (n = 6-16). Standard error bars have been omitted for simplicity. Asterisks designate a significant difference between Low and Control at that age ($p \leq .05$). No statistically significant differences were found between High I and Control at any age sampled.

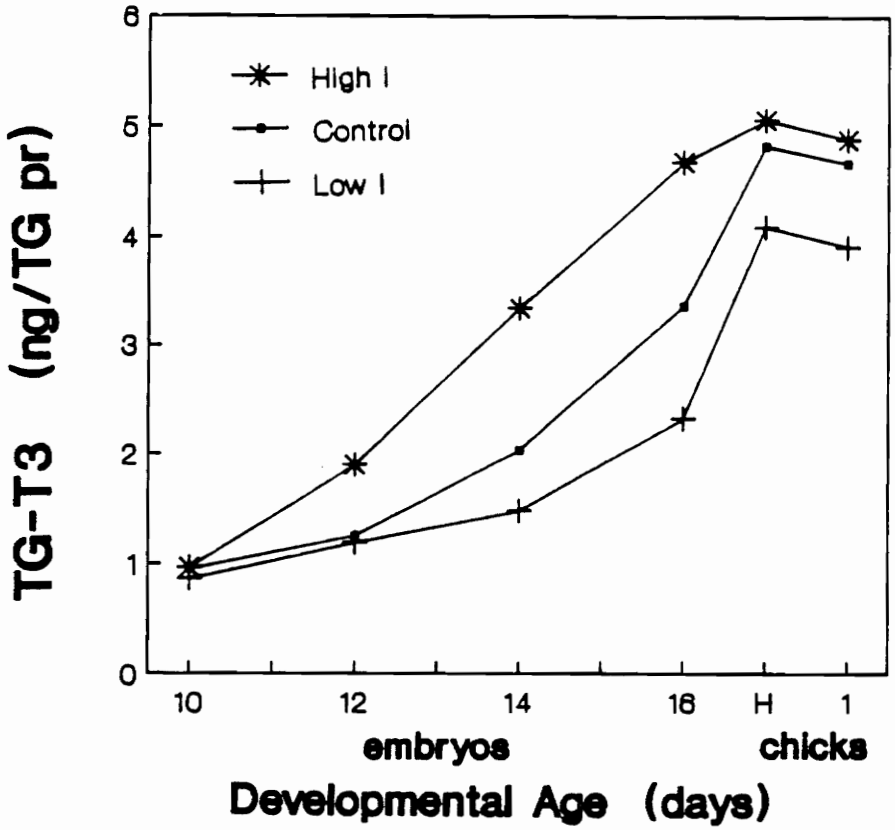

Figure 4. Developmental pattern of thyroid gland T3 content of Japanese quail embryos and chicks from eggs of hens fed diets with different I availabilities. Low I: ≤50 μg/kg feed, control I: 800 μg/kg feed, high I: 1200 μg/kg feed). H designates chicks on the day of hatch. Values are means at each age (n = 6-16). Standard error bars have been omitted for simplicity. No statistically significant differences were found between High I, Low I, and Control at any age sampled.

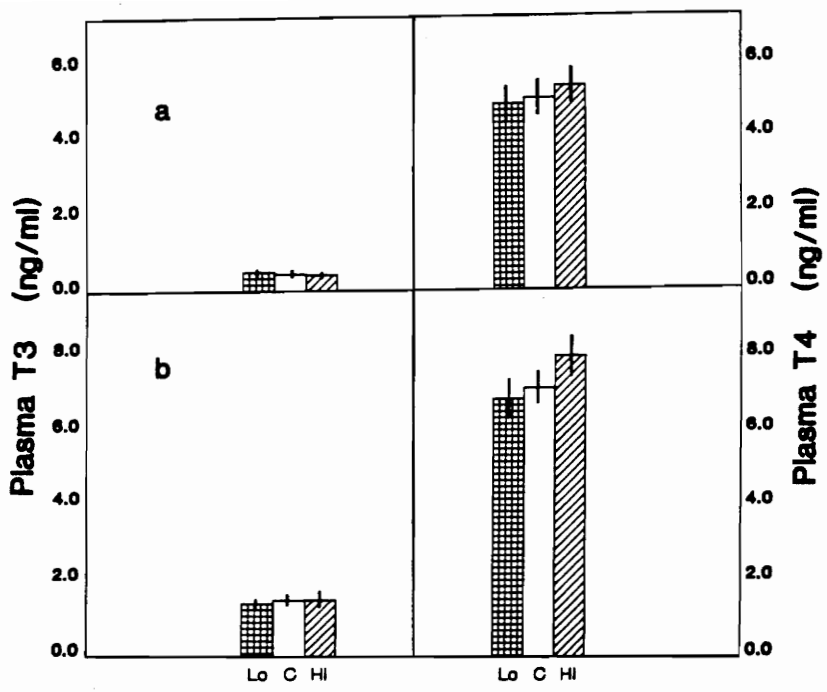

Figure 5. Plasma thyroid hormone concentrations of 14 day embryos (a) and 1 day chicks (b) of Japanese quail from eggs of hens fed diets with different I availabilities. Low I (Lo): ≤50 μg/kg feed, control I (C): 800 μg/kg feed, high I (Hi): 1200 μg/kg feed. Values are means ± 2SE (n = 8-10). Asterisks designate significant differences from control values (p≤.05).

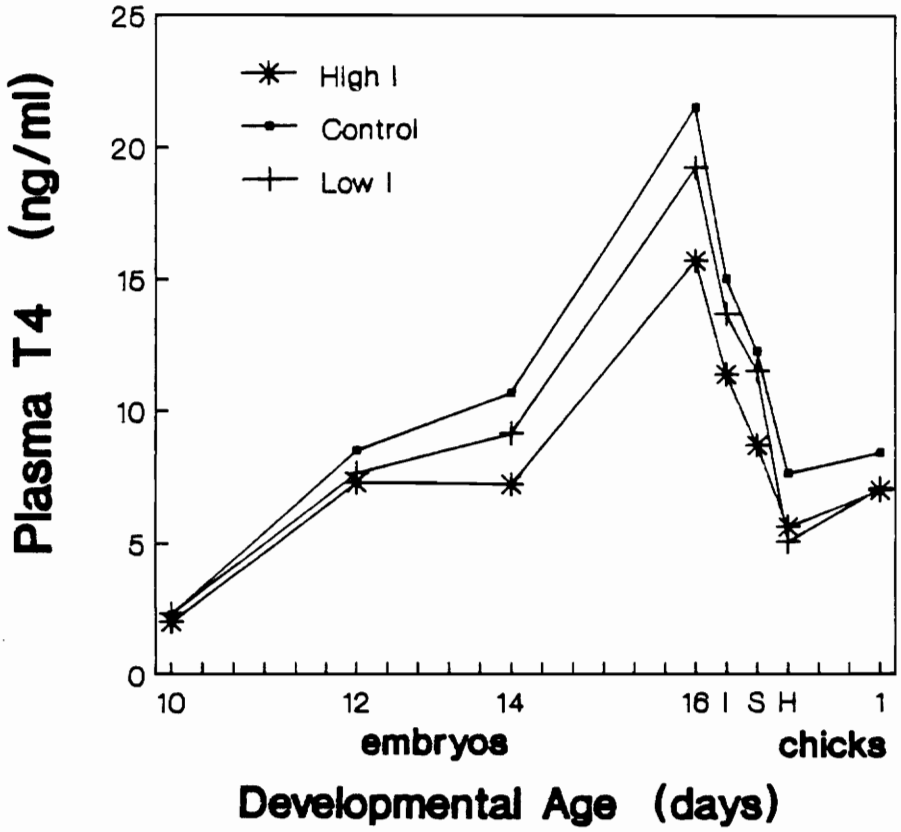

Figure 6. Developmental pattern of plasma T4 concentrations of Japanese quail embryos and chicks from eggs of hens fed diets with different I availabilities. Low I: ≤50 μg/kg feed, control I: 800 μg/kg feed, high I: 1200 μg/kg feed). Perinatal stages are designated as I (penetrated into the air cell), S (pipped through the shell), H (chicks on the day of hatch). Values are means at each age (n = 6-16). Standard error bars have been omitted for simplicity. No statistically significant differences were found between High I, Low I, and Control at any age sampled.

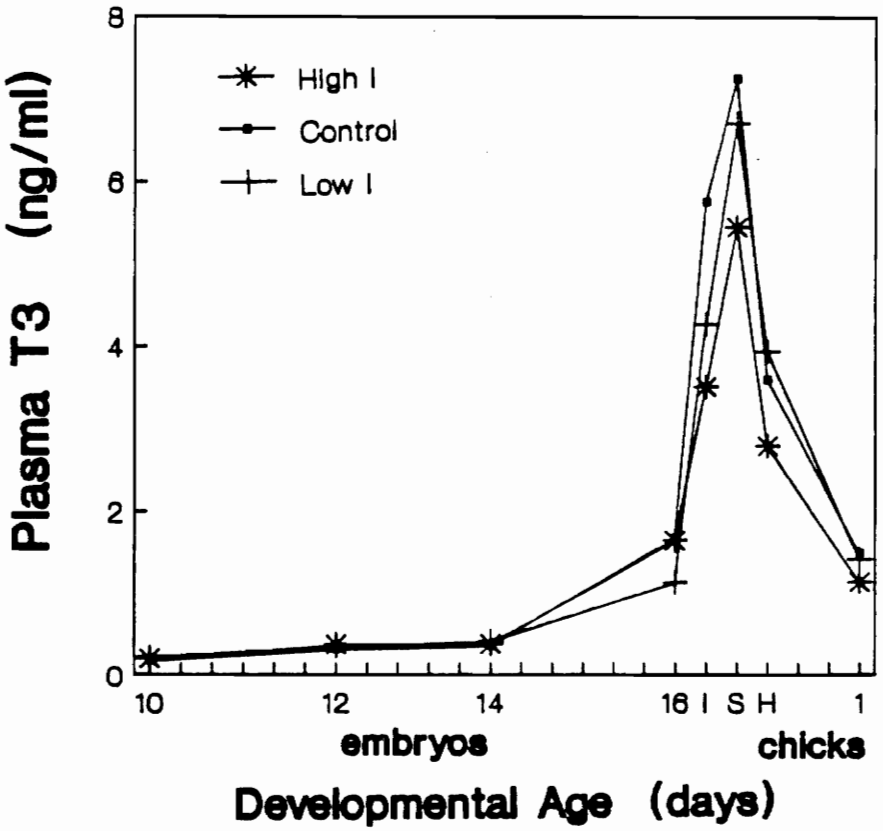

Figure 7. Developmental pattern of plasma T3 concentrations of Japanese quail embryos and chicks from eggs of hens fed diets with different I availabilities. Low I: ≤50 μg/kg feed, control I: 800 μg/kg feed, high I: 1200 μg/kg feed). Perinatal stages are designated as I (penetrated into the air cell), S (pipped through the shell), H (chicks on the day of hatch). Values are means at each age (n = 6-16). Standard error bars have been omitted for simplicity. No statistically significant differences were found between High I, Low I, and Control at any age sampled.

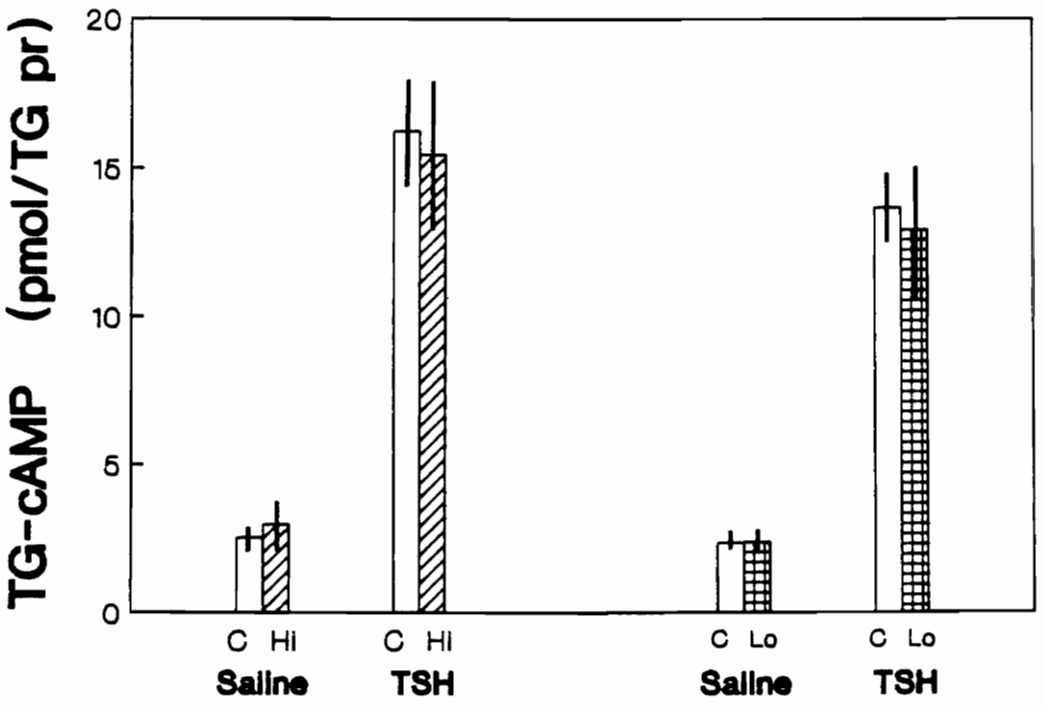

Figure 8. Thyroid gland cAMP response to TSH in 1 day chicks of Japanese quail from eggs of different I availabilities 30 minutes after injection of 25 mU TSH or saline (Sal). Control I (C): 800 μg/kg feed maternal diet , high I (Hi): 1200 μg/kg feed maternal diet, low I (Lo): $\leq$50 μg/kg feed maternal diet. Values are means $\pm$ 2SE (n = 5-15). TSH injected chicks had significantly elevated thyroid gland cAMP compared to saline injected chicks ($p \leq .05$). The magnitude of the response to TSH did not differ between high I and control or low I and control.

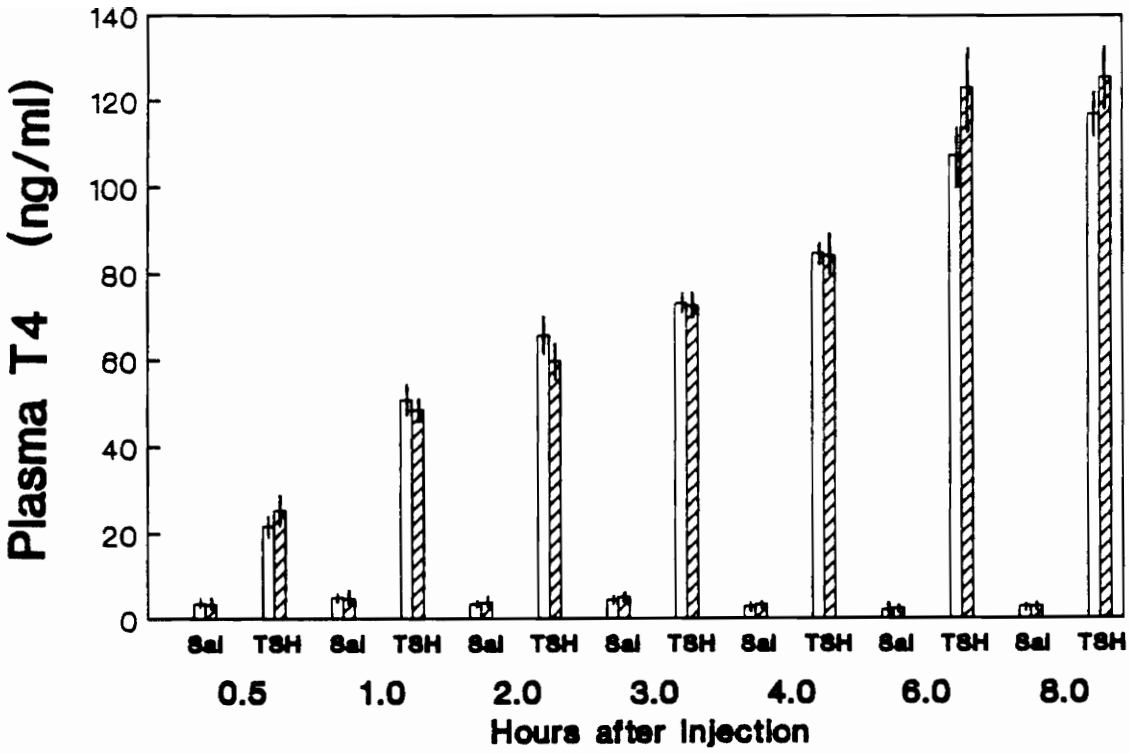

Figure 9. Plasma T4 response to TSH in 1 day chicks of Japanese quail from eggs of different I availabilities at times after injection of 25 mU TSH or saline (Sal). Control I (open bars): 800 μg/kg feed maternal diet , high I (hatched bars): 1200 μg/kg feed maternal diet . Values are means $\pm$ 2SE (n = 5-15). TSH injected chicks had significantly elevated plasma T4 compared to saline injected chicks at all times sampled ($p \leq .05$). The magnitude of the response to TSH did not differ between the I availability groups at any time following injection.

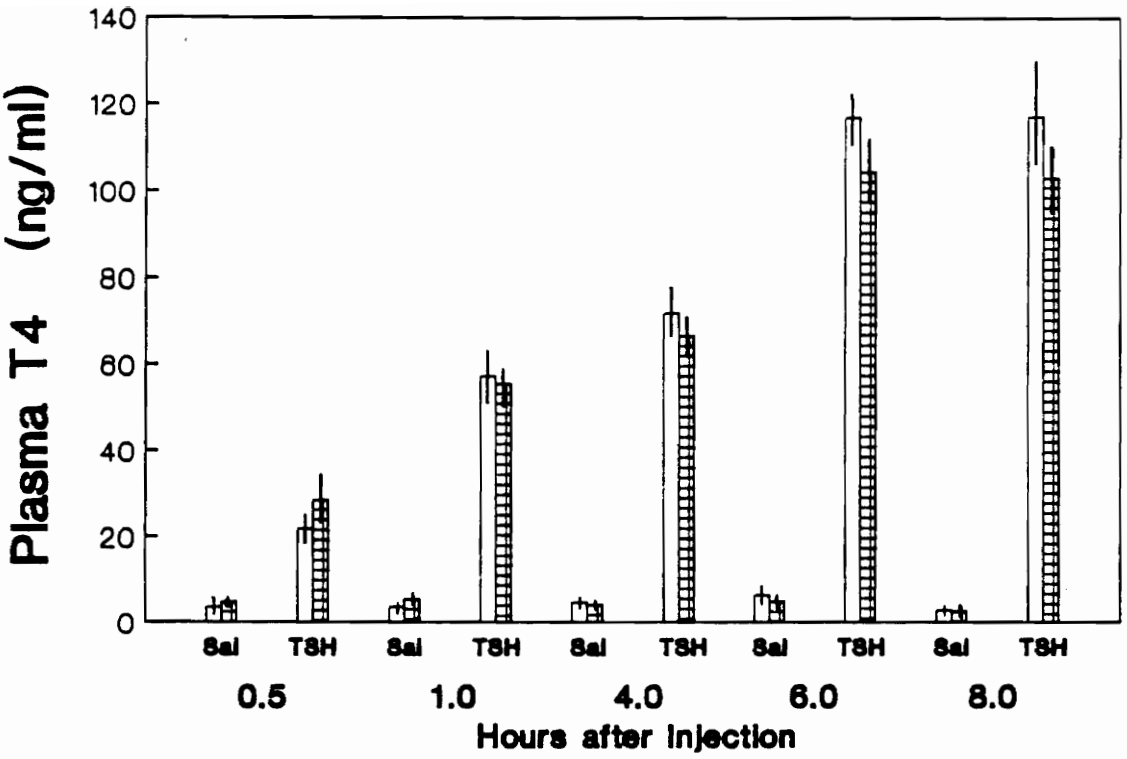

Figure 10. Plasma T4 response to TSH in 1 day chicks of Japanese quail from eggs of different I availabilities at times after injection of 25 mU TSH or saline (Sal). Control I (open bars): 800 μg/kg feed maternal diet , low I (checked bars): $\leq$50 μg/kg feed maternal diet . Values are means $\pm$ 2SE (n = 5-15). TSH injected chicks had significantly elevated plasma T4 compared to saline injected chicks at all times sampled ($p \leq .05$). The magnitude of the response to TSH did not differ between the I availability groups at any time following injection.

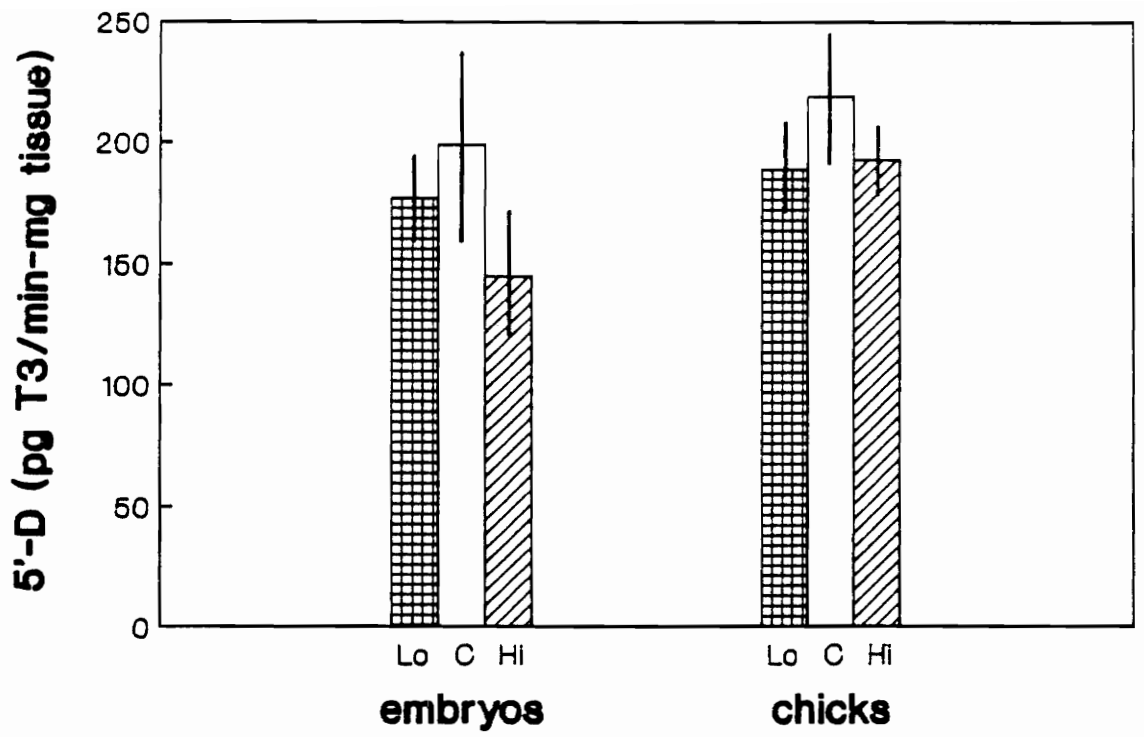

Figure 11. Hepatic 5'monodeiodinase (5'-D) activity of 14 day embryos and 1 day chicks of Japanese quail from eggs of hens fed diets with different I availabilities. Low I (Lo): ≤50 μg/kg feed, control I (C): 800 μg/kg feed, high I (Hi): 1200 μg/kg feed. Values are means ± 2SE (n = 8-10). No statistically significant differences were found between High I, Low I, and Control at either age sampled.

Table 1. The effect of different I availabilities during development on body weight.

	14 d embryos	1 d chicks
High I	3.37 ± 0.30*	4.94 ± 0.39*
Control	4.46 ± 0.35	6.25 ± 0.47
Low I	4.09 ± 0.26	5.92 ± 0.47

Body weight (g) of 14 day embryos and 1 day chicks chicks of Japanese quail from eggs of hens fed diets with different I availabilities. High I: 1200 μg/kg feed, control I: 800 μg/kg feed, low I: $\leq$50 μg/kg feed. Values are means ± 2SE (n = 8-10). Asterisks designate significant differences from control values within a column ($p \leq .05$).

Table 2. The effect of different I availabilities during development on thyroid gland weight.

	14 d embryos	1 d chicks
High I	1.18 ± 0.21	1.36 ± 0.23
Control	1.09 ± 0.18	1.40 ± 0.26
Low I	1.08 ± 0.23	1.50 ± 0.25

Thyroid gland weight (mg) of 14 day embryos and 1 day chicks of Japanese quail from eggs of hens fed diets with different I availabilities. High I: 1200 μg/kg feed, control I: 800 μg/kg, low I: $\leq$50 μg/kg. Values are means ± 2SE (n = 8-10). No statistically significant differences were found between High I, Low I, and Control at either age.

Table 3. Comparison of individuals with thyroid gland hypertrophy with others from the same I availability group.

	14 d embryos		1 d chicks	
	High I	Hypertrophy	High I	Hypertrophy
Wt (mg)	1.16 ± 0.10	3.49 ± 0.29*	1.30 ± 0.13	4.39 ± 0.77*
TG-T4 (ng/TG pr)	185.9 ± 25.3	610.5 ± 72.2*	517.2±124.3	1571.9±395.2*
TG-T3 (ng/TG pr)	2.01 ± 0.45	7.79 ± 2.91*	2.82 ± 0.44	7.87 ± 1.73*
Plasma T4 (ng/ml)	6.25 ± 1.83	2.64 ± 0.56*	9.22 ± 1.25	3.36 ± 1.24*
Plasma T3 (ng/ml)	0.53 ± 0.19	0.21 ± 0.07*	1.49 ± 0.32	0.29 ± 0.27*
	(n = 56)	(n = 5)	(n = 42)	(n = 4)

Thyroid gland weight (Wt), thyroid gland T4 and T3 content (TG-T4, TG-T3) and plasma T4 and T3 concentrations of 14 day embryos and 1 day chicks of Japanese quail from eggs of hens fed a high I diet (1200 μg/kg feed). Values means ± 2SE. Asterisks designate significant differences within an age group ($p \leq .05$).

Discussion

Adult Japanese quail have excellent ability to maintain plasma T4 and T3 concentrations over a wide range of dietary I availability (McNabb et al., 1985a). Likewise, in the present study, plasma hormone concentrations and the developmental pattern of TH are not altered despite different I availabilities in the egg. Embryos maintained "normal" plasma TH with reduced or elevated I availability as early as day 10 of incubation, shortly after the TG becomes functional. The perinatal peak in TH that is associated with hatching also is unaltered despite differences in I availability. This result confirms the few previous reports in embryos (McNabb et al., 1985b) and chicks (Newcomer, 1978; McNabb et al., 1985b) and extends our knowledge concerning the ability of the developing embryo's TG to maintain plasma TH concentrations throughout development with different amounts of I available in the egg.

Regulation of thyroid function with high I availability

With increased I availability, TG-I is elevated in quail embryos and chicks. Similar findings have been reported previously (chickens: Newcomer, 1978; quail: McNabb et al., 1985b). However, TG-I

is not elevated in adult Japanese quail with moderately increased I availability (McNabb et al., 1985a). Evidently, the TG of developing birds does not regulate I content in the same manner as adult birds.

TG-TH content, weight-specific TG-TH, and the developmental pattern of TG-TH are not altered with increased TG-I. Studies of mammalian Tg suggest there are a limited number of hormonogenic residues within the Tg molecule. The plateau in TG-TH at high levels of I found in the present study may indicate all hormonogenic residues have formed TH. Thus, beyond a certain level, increased TG-I may not result in increased TH formation. In the present study, support for this idea comes from those individuals that exhibit TG hypertrophy with increased I availability. In these individuals, total TG-TH content is elevated but weight specific TG-TH is not increased. This suggests that the increased TH content of these individuals is a result of the enlargement of the gland which presumably results in increased amounts of Tg. This phenomenon, increased TG weight and TG-T4 content with high I availability, has been reported in juvenile chickens (Suzuki et al., 1969), but these data were not expressed on a weight-specific basis. However, it appears that most of the increases in TG-T4 reported by these researchers was due to increased TG size as in the present study. Other factors such as peroxidase or hydrogen peroxide, could explain the maintenance of TG-TH at control levels despite increased I content.

In the present study, the control group was reared from eggs of hens fed a diet containing 800 μgI/kg feed, a common I supplementation for gamebird feed. Previous research (McNabb et al., 1985a) indicates that I supplementation of 150 μgI/kg feed is sufficient for normal thyroid function in adult Japanese quail. The present study also indicates that the I availability of gamebird feed is well in excess of I required for TH synthesis for developing quail.

The TG's ability to respond to TSH is not altered by increased I availability and TG-I content. Following TSH stimulation, TG content of cAMP, the predominant second messenger in the TG, is not altered with increased TG-I in comparison to the TSH response of controls. The increase in plasma TH concentrations in response to TSH also is not affected by elevated TG-I. In contrast, TSH-induced TH release is reduced in rats exposed to elevated I availability (see review by Nagataki

and Ingbar, 1986). Thus, birds, unlike mammals, may regulate thyroidal dynamics without altering the response of the gland to TSH stimulation.

Increased I availability had no effect on hepatic 5′-D activity and plasma TH concentrations, which suggest that peripheral TH dynamics are not changed. Other investigators have concluded that the peripheral turnover of radiothyroxine is not altered by increased I availability (Suzuki et al., 1969). However, this conclusion is based on the disappearance of radioiodine in the plasma, an inaccurate estimate of radiothyroxine, because it does not separate radiothyroxine from other labelled breakdown products. It appears that peripheral hormone metabolism is not altered with elevated I, but more extensive research is needed to verify this possibility.

Elevated I availability was associated with reduced body weight in the present study. Similar results have been reported by some investigators (chickens: Mayberry and Hockert, 1968) but not others (chickens: Newcomer, 1978; quail: McNabb et al., 1985b). The reduced weight may be due to alterations in other growth factors or subtle changes in thyroid dynamics which were not detected. Although plasma TH and hepatic 5′-D activity were not altered, measurement of tissue TH concentrations and peripheral hormone turnover are needed to be certain that the reduced growth is caused by changes in thyroid dynamics due to the increased I availability.

In general, excess I does not alter TG weight. Similar findings have been reported (Mayberry and Hockert, 1968; Newcomer, 1968, 1978). However, in the present study, a small percentage (9%) of the birds exposed to increased I availability did exhibit marked hypertrophy of the TG. Increased TG weight due to excess I has been reported in birds (Wheeler and Hoffman, 1949; Suzuki et al., 1969; Takeuchi et al., 1970; McNabb et al., 1985b). In the present study, individuals with TG hypertrophy have elevated TG-TH content but reduced plasma TH concentrations. Evidently, these birds have some inadequacy in TH release resulting in low plasma TH which in turn caused increased TG weight due to elevated TSH stimulation.

With increased I availability during development, most aspects of thyroid function are not changed. Regulation occurs at the level of TH synthesis despite elevated TG-I content. Thus, other factors involved in thyroidal dynamics need not be altered to maintain plasma TH concentrations.

Regulation of thyroid function with low I availability

With reduced I availability during development, reduced TG-I has been a consistent finding (this study; Creek et al., 1957; Rogler et al., 1959a, 1961b; Newcomer, 1978; McNabb 1985b). Reduced TG-I results in an increased T3/T4 ratio within the gland. Increased T3/T4 ratios are reported in adult quail fed a diet low in I (McNabb et al., 1985a) and presumably are due to an increased MIT/DIT ratio which has been found in chickens with low I availability (Rosenberg et al., 1963). In the present study, TG-T3 content was maintained throughout development; reduced T4 content causes the altered ratio. Thus, with lowered I availability, storage of T3, the metabolically active hormone, is maintained.

Because T3/T4 ratios within the gland are increased with reduced I availability, the T3/T4 ratio of TH released from the gland also may be elevated. However, plasma T3/T4 ratios are not different from control values. Although these studies suggest that 5′-D content of the tissues is not altered with reduced I availability, the *in vivo* rates of deiodinase activity or of TH degradation/excretion may be changed to achieve the maintenance of plasma TH ratios with altered TG-TH stores.

Reduced TG-I does not change the gland's response to TSH; TG-cAMP and plasma TH concentrations were not different following TSH stimulation with low I availability versus controls. Consistent with this finding, reduced I availability does not alter the perinatal peak in plasma T4 concentrations in quail, a phenomenon that in chickens is caused by endogenous TSH stimulation (reviews by Thommes, 1987, 1988).

Reduced I availability did not affect body weight or TG weight in this study. Other researchers have found no change in TG weight or body weight with low I availability (60-125 μgI/kg chick feed or maternal diet: Rogler et al., 1961b: Newcomer, 1978; McNabb et al., 1985b). In contrast, reduced growth and TG hypertrophy are reported in embryos and chicks with severe I deficiency (< 35 μgI/kg feed or maternal diet: Creek et al., 1957; Rogler et al., 1959a, 1961b). Therefore, I would term ≤ 50 μgI/kg feed in the maternal diet, as used in the present study, a moderate but not extreme I deficiency for developing Japanese quail. This level of dietary I also is considered low for

mammals (rats); < 20 μgI/kg feed is considered a severely deficient diet (see review by Taurog, 1986).

Overall, developing Japanese quail exhibit excellent ability to regulate their thyroid function over a wide range of I availabilities. The key regulation appears to occur at the level of TH synthesis. Thus, with excess I, TH formation is restricted to that characteristic of controls; with I deficiency, TH formation is altered to maintain synthesis of T3, the metabolically active hormone despite an inability to maintain T4. These adjustments in TH formation, allow most aspects of TH dynamics to remain unchanged and circulating hormone concentrations are "normal" throughout development, despite different I availabilities.

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1986 summer stipend, VPI&SU (awarded as a competitive scholarship to six first year students on progress of thesis project). $1,000.

1986 fall, Tuition Scholarship (awarded by Graduate Committee based on progress toward degree).

FUNDED GRANTS:

1986 Sigma Xi Grants-in-Aid of Research. The Effects of high iodine on thyroid function during development in Japanese quail. $300.

1986 Funds to match Grants-in-Aid of Research, Department of Biology, VPI&SU. $300

1987 Virginia Academy of Science. The Effect of different iodine availabilities on the responses of the thyroid to thyrotropin during development in Japanese quail. $500.

1987 Funds to match Virginia Academy of Science, Department of Biology, VPI&SU, $500.

MEMBERSHIP IN PROFESSIONAL ORGANIZATIONS:

Virginia Academy of Science

American Society of Zoologists

Sigma Xi, The Scientific Research Society

PAPERS PRESENTED AT SCIENTIFIC MEETINGS:

Stallard, Lana C. and F.M.A. McNabb (1986). The Influence of iodine on hormone production by the thyroid glands of quail. Va. J. Sci., 37, 72.

Stallard, Lana C. (1986). Thyroid hormones and peripheral deiodination with high iodine availability in Japanese quail. 3rd Annual Graduate Student Symposium.

Stallard, Lana C. and F.M.A. McNabb. Thyroid gland function in developing Japanese quail with different iodine availabilities. For presentation at the Eastern Regional Conference on Comparative Endocrinology, May 1987, Rutgers University.

Freeman, T.B., L.C. Stallard, M.J. McNichols and F.M.A. McNabb. Evaluation of methimazole (MMI)-induced hypothyroidism in adult Japanese quail. Presented at the Eastern Regional Conference on Comparative Endocrinology, May 1987, Rutgers University.

Freeman, T.B., M.J. McNichols, L.C. Stallard and F.M.A. McNabb (1987). 5'deiodinase (5'D) activity in methimazole (MMI)-induced hypothyroidism in Japanese quail. Amer. Zool. 27:78A. Presented at National meeting of American Society of Zoolologists, December, 1987, New Orleans, LA.

Stallard, Lana C., F.M.A. McNabb, Thyroidal and Peripheral Regulation with Differing Iodine. For presentation at the Eastern Regional Conference on Comparative Endocrinology, May 1988, Franklin and Marshall College.

First authorship indicates presentations made by L.C. Stallard

WORK IN PROGRESS

Stallard, L. C. The Effects of Different Iodine Availabilities on Thyroid Function during Development in Japanese quail. Manuscript from thesis to be submitted to Domestic Animal Endocrinology.

Studies with F.M.A. McNabb, A. Dunnington, and P. Siegel: "Peripheral Thyroid Function and Resource Partitioning during Growth and Development ".