THYROID HORMONE ACTIVATION BY INTESTINAL TISSUE OF HIGH AND LOW WEIGHT-SELECTED CHICKENS

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ABSTRACT

The objective was to study the enzymatic production of triiodothyronine (T3) in the intestine of chickens during perinatal intestinal maturation in two lines of chickens selected for high (HW) or low (LW) body weight at eight weeks of age. Valid assay conditions (proportionality of enzyme activity with enzyme concentration and assay time) were established and the intestinal 5'-deiodinase (5'D) activity was characterized for comparison with other tissues. The characterization studies showed that intestinal 5'D is like the Type I 5'D in liver of birds and mammals previously studied. Specific activity of adult intestinal 5'D is significantly higher in the HW than in the LW line. In both lines intestinal 5'D increases significantly between embryos that have not pipped into the air cell (NP) and embryos that have pipped into the air cell (AC) and 5'D activity peaks in embryos that have pipped through the shell (TS). In contrast to the line differences in adults, LW embryos have much higher 5'D specific activity than HW embryos until 1d posthatch. Plasma thyroxine (T4) and T3 also increased between consecutive stages and peaked in embryos pipped through the shell, then decreased abruptly at 1d posthatch. Both plasma hormones were higher at each perinatal stage in the LW line than in the HW line and the LW line hatched earlier than HW. Intestinal alkaline phosphatase (a marker of differentiation)
showed a significant increase in activity at each of the stages of development in both lines. Alkaline phosphatase activity was significantly higher in the LW line than the HW line at the NP, AC and TS stages but not at 1d posthatch. Previous work in other laboratories indicates that T3 plays a role in triggering intestinal differentiation and maturation of intestinal function for posthatching life. The results of this study indicate that T3 for this signal originates at least partially from 5'deiodination of T4 within the intestinal tissues as well as from T3 available in the plasma.
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INTRODUCTION

Thyroid hormones are necessary for body and organ development of birds and mammals, but less is known about the role thyroid hormones play in development of individual tissues. Thyroid hormones influence cell differentiation in the gut of embryonic chickens. Black and Moog (1978) show gut epithelial cells hypertrophy, increase in alkaline phosphatase content and increase their capacity for sugar transport when cultured embryonic intestinal pieces are subjected to T4 treatment. T3, the deiodination product of T4, directly induces intestinal enzymes involved in digestion and enhances glucose transport across the intestinal wall (Black, 1988; Black and Moog, 1978). T3 also stimulates D-glucose uptake by intestine through induction of a low affinity transport system (Black, 1988; Prager et al., 1990).

Two lines of chickens which were genetically selected for high or low body weight from a single base population, were used to examine production of the active thyroid hormone, T3, during intestinal development. In the high weight (HW) line, during late embryo and early posthatching life, rapid growth of the intestine (a "supply" organ) is associated with later final growth of tissues such as skeletal muscle (a "demand" organ). However, the LW line hatches earlier than the HW line so the intestine may function in posthatching food assimilation at an earlier age in this line than in the LW line.

This study considered 5'-deiodination of T4 to T3 in intestine of high and low weight lines of chickens, at the perinatal period of development. The objectives in this study were (1) to validate 5'D assay conditions in adult HW and LW lines of chickens, since our study was the first to examine 5'deiodination in intestine; (2) to use these validated conditions and characterize this intestinal enzyme, allowing for comparison to previous avian and
mammalian studies in other tissues; and (3) to study at embryonic, perinatal and post-hatching stages, developmental patterns of 5'deiodinase (5'D) enzyme activity, and alkaline phosphatase activity, in relation to plasma thyroid hormone concentrations.
LITERATURE REVIEW

Thyroid Physiology

Thyroid hormones exert a powerful and important regulatory influence over body growth and development. They also play an important role in tissue growth and differentiation, metabolism, reproduction and thermogenesis in vertebrates. Experiments have shown that thyroid hormones, together with growth hormone and other growth factors, regulate growth, differentiation and maturation of cells in various tissues during development in birds and mammals (King and May, 1984; Legrand, 1986; McNabb and King, 1993; Schwartz, 1983). In mammals, thyroid hormones, unlike growth hormone, cannot act alone to stimulate growth, but are essential in physiological concentrations for normal organ and body growth and maturation.

The thyroid gland produces two principal products, the prohormone- thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3). Under normal conditions T4 is synthesized and circulated in greater quantities than T3, which is the active form of the thyroid hormone that binds to nuclear receptors and triggers physiologic response in target tissues (Oppenheimer et al., 1987). The activation of T4 to T3 is catalyzed by the 5'deiodinase (5'D) enzyme in extra-thyroidal tissues. The T3 is released into the plasma, whereupon it is transported to target tissues. In liver, kidney and skeletal muscle, 80-90% of the peripheral deiodination of T4 to T3 is produced by 5'D (Engler and Burger, 1984).

Plasma proteins transport 99% of the thyroid hormones in blood. Free thyroid hormones
in plasma are in equilibrium with bound hormones and are replaced as the hormones are metabolized. Thyroxine is more tightly bound to plasma proteins than T3 and this allows for faster clearance of T3 from circulation. It also partly explains how T3 readily enters the target cells.

The secretion of thyroid hormones into blood is regulated by the hypothalamic-hypophyseal-thyroid axis, which is affected by environmental factors such as temperature and photoperiod, and by internal changes in nutritional status (Sterling and Lazarus, 1977). Thyroid hormone production and secretion are regulated by thyroid stimulating hormone (TSH) released by the hypophysis, which in turn is regulated by thyrotropin-releasing hormone (TRH) from the hypothalamus. Production and secretion of TRH and TSH are modulated by negative feedback of thyroid hormones on the pituitary gland and hypothalamus (Norris 1985a).

The thyroid gland, paired in birds and bilobed in mammals, which produces thyroid hormones is adjacent to the trachea. It is surrounded by a connective tissue sheath and has numerous follicles, with a capillary network surrounding each follicle. Each thyroid follicle is a single layer of epithelial cells surrounding a colloid-filled lumen. Thyroglobulin, a glycoprotein, is synthesized by the follicular cells and stored in the lumen of the follicles. Inorganic iodide atoms are bound to tyrosine residues of the thyroglobulin in the follicular cells. The final stage of synthesis of thyroid hormones is the coupling of two of these iodinated tyrosines in the thyroglobulin. The formation of T4 or T3 depends upon the coupling of one monoiodinated tyrosine (MIT) with a diiodinated tyrosine (DIT) or two diiodinated tyrosines together. Hydrolysis of thyroglobulin molecules by epithelial cells leads to release of thyroid hormones into the circulation (Gorbman et al., 1983; Norris, 1985a).
Mechanism of action on TH receptors

Secretion of T4 by the thyroid gland and circulating levels of T4 in the plasma are much greater than T3. Most of the circulating T3 is produced by peripheral deiodination. Presence of nuclear T3 receptors has been used to support the idea that T3 is the physiologically active TH. Oppenheimer's study suggests that free T3 in the plasma readily crosses the plasma membrane to reach nuclear receptor sites (Oppenheimer et al., 1987).

Triiodothyronine readily enters the nucleus of target cells and binds to chromatin sites initiating gene transcription. By production of mRNA, it influences protein synthesis (Oppenheimer et al., 1987). Proteins synthesized could be enzymes that regulate metabolic activities in the cell, or peptide hormones that regulate activities of other tissues (Oppenheimer, 1989). Triiodothyronine can affect oxidative phosphorylation in mitochondria (Sterling and Lazarus, 1977). Nuclear receptors for THs have a greater affinity for T3 than T4 (Oppenheimer, 1979). High affinity, low capacity binding sites for T3 have been shown in liver, kidney, brain, heart, spleen, lungs and anterior pituitary by Oppenheimer (Oppenheimer et al., 1972; Oppenheimer, 1983). TH nuclear receptors of both the alpha and beta forms, coded by two different genes, have been found in rat and man (DeGroot et. al., 1989). Triiodothyronine nuclear receptors were found in most vertebrate classes (birds: Bellabarba et al., 1988; amphibians: Galton, 1988b; fish: Weirich et al., 1987) and appear to be conserved evolutionarily.

Physiological actions of THs

Actions of THs are varied. Some effects are direct, for example, T3 receptor regulation of protein synthesis. THs also have indirect or permissive effects, in which they act synergistically with other hormones, or prime a cell or tissue to the action of another
hormone. Actions of THs generally can be put into two categories: (1) growth and differentiation effects, and (2) metabolic effects.

1. Thyroid hormone effect on growth and differentiation.

Thyroid hormones influence both growth and differentiation during prenatal and postnatal life. Growth, as defined by Legrand (1986), is an increase in cell size and number. Maturation (differentiation) is a complex change in progressive diversification of cell structures and functions that lead to the adult organism. Growth and maturation are the two components that constitute development.

Thyroid hormones influence the development of all vertebrate classes. One of the first studies to recognize this was on amphibian metamorphosis (Gudernatsch, 1912). Tadpoles underwent early metamorphosis when fed mammalian thyroid tissue. Thyroidectomized or goitrogen treated tadpoles grew in size, but did not metamorphose (Allen, 1932; Weber, 1967).

In birds and mammals, THs play an important role in the development of: central nervous, skeletal, reproductive, and pulmonary systems; the intestine; pelage and plumage (Legrand, 1986). Thyroid hormones are important for critical events of development of the brain. Thyroid hormone deficiency results in abnormal development and functioning of the central nervous system, thus causing impaired mental development in humans (Norris, 1985a). During the latter part of brain development, deficiency of THs affect migration of granule cells in the cerebellum, and reduce the number of glial cells, myelin content, and axonal density (Nunez, 1984; Schwartz, 1983).

For normal postnatal growth and development, THs interact synergistically with growth hormone to cause increased body weight, skeletal size and muscle growth (King and May,
Maturation of lung tissue is dependent on the presence of THs during perinatal periods for birds, and the fetal stages for mammals (Whittmann et al., 1984). Other tissues requiring THs for functional maturation are the reproductive system (Legrand, 1986) and gastro-intestinal tract. Induction of sugar transport mechanisms, and triggering of intestinal enzymes are a consequence of TH effects (Black, 1988; Black and Moog, 1978; Prager et al., 1990).

2. Metabolic effects
The action of THs on metabolism can be seen by the clinical symptoms of hypothyroidism and hyperthyroidism in humans. Hypothyroidism is characterized by hypophagia, low basal metabolic rate and decrease in oxidative enzymes; patients with hyperthyroidism show the opposite (Norris, 1985a).

In mammals, THs play a role in thermogenesis and maintenance of constant body temperature, as well as producing changes in carbohydrate, protein and lipid metabolism. Studies in mammals indicate that brown adipose tissue (BAT) is an important thermogenic tissue in small hibernators and is present in infants soon after birth. The high amounts of heat production required for arousal from hibernation are produced by BAT, which is innervated by the sympathetic nervous system. There is an increase in peripheral deiodination of T₄ by BAT and this results in increased T₃ release to plasma with increased cold exposure. Uncoupled mitochondrial respiration in BAT is responsible for the high amounts of heat produced in response to cold exposure (Silva and Larsen, 1983).

During their calorigenic or heat-generating actions in response to cold stress, THs accelerate glucose oxidation, and basal metabolic rate (BMR) which causes an increase in oxygen consumption (Norris, 1985a; Sterling and Lazarus, 1977). In addition to glucose oxidation, THs increase mitochondrial oxidative activity which decreases ATP synthesis in
mitochondria and thereby increases glucose oxidation and heat production (Guernsey and Edelman, 1983; Norris, 1985a).

Adaptation to long term cold stress in mammals causes increased synthesis of mitochondrial respiratory proteins, cytochrome C, cytochrome oxidase and succinoxidase by THs (Bianco and Silva, 1987; Guernsey and Edelman, 1983; Oppenheimer, 1983). Thyroid hormones may have permissive effects, with hormones like epinephrine and glucagon, on carbohydrate metabolism by increasing glycogenolysis in the liver and increasing lipolysis. Depending upon the condition and type of tissue under examination, THs play either an anabolic or catabolic role during nitrogen metabolism. Thyroid hormones stimulate tissue growth and protein content when administered in low doses, but have a catabolic effect, especially in the breakdown of muscle, when administered in high doses (Gorbman et al., 1983; Norris, 1985a).

Peripheral deiodination of thyroid hormones

Only one-seventh to one-half of the circulating T3 is produced by the thyroid gland. Most T3 is produced by peripheral deiodination of T4. The amount of T3 produced by deiodination of T4 in man is estimated to be 80% (Engler and Burger, 1984). This deiodination is accomplished largely by organs such as liver and kidney. When produced by organs such as brain and pituitary, T3 remains largely within the tissue (Van Doorn et al., 1984).

Deiodination Pathways

Deiodination can occur at either the inner ring or the outer ring. When an iodine is removed at the 5' position of the outer ring of T4, it results in the formation of the physiologically active T3, this being the activating pathway. T4 can also undergo a 5'deiodination of the inner ring resulting in the formation of rT3. This formation of rT3 is the deactivating
pathway since rT3 has no known physiological function. These products can undergo further deiodinations in the thyroid gland, resulting in iodinated tyrosines (MIT and DIT); the inorganic iodide released is used for iodination of newly synthesized thyroglobulin (Engler and Burger, 1984; Hesch and Koehrle, 1986).

Investigations with rat microsomes from different tissues led to the identification of three pathways or isoymes of 5'D with different biochemical and physiological properties (Hesch and Koehrle, 1986; Kaplan, 1986; Leonard and Visser, 1986). Studies done with cellular fractions indicate that all three deiodination pathways are present only in microsomes, specifically within the endoplasmic reticulum (Balsam et al., 1979; Kaplan, 1979; Silva and Larsen, 1986). The exception is the brain where 5'D is located in the plasma membrane in neurons (Hesch and Koehrle, 1986). The three types of isoymes are classified according to these characteristics: deiodination sites (whether inner or outer ring deiodination), substrate preference (T4, T3, or rT3), Michaelis constant (Km), tissues where they are found, kinetic mechanisms and sensitivity to 1 mM propylthiouracil (PTU) (Kaplan, 1986; Leonard and Visser, 1986) and 1 mM iopanoic acid (IOP).

Type I deiodinase pathway
This pathway is characteristic of the liver, kidney and thyroid gland. It is capable of both 5'-deiodination of T4 to T3, and also 5'deiodination of the inner ring of T4 to rT3. Little is understood about what conditions favor one reaction over the other, and it has been assumed that a single enzyme catalyzes both reactions (Hesch and Koehrle, 1986; Leonard and Visser, 1986). Type I deiodinase has a higher affinity for rT3 than T4, which has led to the idea that one of its functions is to dispose of rT3. Type I has a Km for T4 of ~ 1 μM and is completely inhibited by PTU and IOP. Originally, inhibition by 1 mM PTU was the only criterion used to distinguish Type I from Type II pathways, but now the other criteria also are used to distinguish the pathways (Silva et al., 1987).
The Type I pathway is stimulated by thiol cofactors; the commonly used cofactor for in vitro studies is dithiothreitol (DTT). 5'-D contains sulfhydryl groups which are oxidized during deiodination and subsequently reduced to their original form. Cells contain abundant quantities of a non-protein sulfhydryl compound, glutathione (GSH). Experiments have shown that there is an inverse correlation between oxidized glutathione and 5'D activity. It has been proposed that the ratio of reduced to oxidized glutathione may regulate 5'D activity (Leonard and Visser, 1986; Sato et al., 1983).

Type II deiodinase pathway

Type II deiodinase is found in the central nervous system, pituitary, and brown adipose tissue. It catalyzes 5'-deiodination of T4 to T3 and rT3 to T2. Type II deiodinase prefers T4 as a substrate and is referred to as a "true T4 deiodinase". Type II also differs from Type I in having a Km for T4 in the nanomolar range (~1 nM), a much higher thiol requirement, and it is inhibited by IOP but not by PTU. Type I and Type II pathways also differ in product destination, i.e., T3 is exported to blood from Type I pathways, but T3 produced by Type II is used locally within tissues where it is produced. The exception is BAT, where Type II pathways provide T3 for release to the circulation (Silva and Larsen, 1986).

Type III deiodinase pathway

Type III deiodination is found mainly in brain and placenta. Type III catalyzes an inner ring 5-deiodination, prefers T3 as a substrate over T4, and has a moderately high Km (~40 nM). Type III is stimulated by thiols and is inhibited by IOP. Type III activity is insensitive to PTU (Leonard and Visser, 1986). Type III functions as a deactivating pathway through which T4 is converted to rT3 or T3 is degraded to T2 (Kaplan, 1986; Leonard and Visser, 1986).
Molecular characterization of Type I and Type II deiodinase

It has taken at least a decade for studies on purification and identification of protein structure of deiodinase enzymes to show that the type I enzyme has a molecular mass of approximately 55 kDa. Cloning studies proved successful and revealed that the type I enzyme is a selenoprotein which contains selenocysteine at the active site. The proposed mechanism by which the enzyme reacts with T₄ is as follows. The selenolate anion of selenocysteine is the iodide acceptor in the active center of the enzyme. The enzyme releases T₃ by reacting with T₄ to remove the 5′-iodide. The enzyme then is changed back to its original form by reacting with a thiol cofactor to release the iodide and revert back to the free enzyme form. PTU acts as an inhibitor by competing with thiol for the selenoyl iodide and forms the inactive selenoyl-thiol complex (Berry and Larsen, 1992). Nutritional studies have found that rats maintained on a diet devoid of selenium had higher levels of plasma T₄, lower levels of plasma T₃ and a marked decrease of hepatic 5′D activity (Beckett et al., 1987).

It has been suggested that the type II deiodinase enzyme may contain cysteine instead of selenocysteine in the active site. Sedimentation studies have shown that it has a molecular mass of approximately 199 kDa (Berry and Larsen, 1992). Nutritional studies in hypothyroid, selenium-deficient rats showed that type II activity in the brain did not differ from control rats supplemented with selenium (Chanoine et al., 1991).

Avian deiodinase studies

Most of the initial deiodinase studies were done in mammals, and avian studies used the same conditions previously developed in the mammalian studies. Up to 1991, Galton and
Hiebert (1987), Rieman and McNabb (1990), and Freeman and McNabb (1991), were the few avian studies that used validated assay conditions for 5'D activity to demonstrate initial velocity conditions. It is essential that validated studies be carried out when measuring enzyme activity. In addition, if measurements of 5'D are to be compared among classes and species, as well as among different treatments, the studies first must demonstrate that enzyme activity is proportional to enzyme concentration and linear with assay incubation time (Dixon and Webb, 1979; Engel, 1981).

Information based on validation studies shows that avian deiodinases are similar to mammalian deiodinases in their characteristics, as well as in responses to physiological and environmental changes (Decuypere and Kuhn, 1988; Galton and Hiebert, 1987; McNabb et al., 1986; McNabb and Freeman, 1990; Rudas, 1986). By comparison of avian hepatic 5'D to mammalian 5'D, it has been found that the enzyme is a Type I enzyme with characteristics comparable to mammalian hepatic 5'D (Freeman and McNabb, 1991; Galton and Hiebert, 1987; Rudas, 1986).

Increased metabolic demands soon after hatching show a shift from inactivating pathways of TH metabolism, to activating pathways during late embryonic stages (Borges et al., 1980, 1981; Freeman and McNabb, 1991; Galton and Hiebert, 1987). This is seen in precocial birds (e.g., chicken and quail), where the young lead independent lives shortly after hatching. Hepatic 5'D activity was measured during development (day 12 in quail: Freeman and McNabb, 1991; and day 15 in chickens: Galton and Hiebert, 1987). These studies suggest that hepatic 5'D activity may be the major source of plasma T₃. These studies also have found good correlation between plasma T₃, T₃/T₄ ratios and hepatic 5'D (Freeman and McNabb, 1991; McNabb, 1988). The perinatal peak of THs may be related to initiation of thermoregulatory capacities in precocial birds (McNabb, 1988).
Avian intestinal studies

Intestine of the chick embryo undergo an important morphological change about a week before hatching. On day 14, there are 16 previlous ridges that run longitudinally along the luminal surface, but no villi can be seen. Soon after (day 15-17), an indentation occurs with formation of the villi. Increased epithelial differentiation occurs around day 18 with subsequent increases in height of the epithelial cells, leading to a functionally mature intestine by two days posthatch. Few microvilli are present at day 14; microvilli increase in density during the last few days prior to hatching (Coulombre and Coulombre, 1958). Functional ontogeny accompanies changes in enzyme activities and transport capacities (Deren et al., 1965; Moog, 1950). In vitro studies indicate that a sugar transport mechanism in intestine is active during the three days prior to hatching and develops peak function about two days posthatch (Bogner and Haines, 1964).

Thyroid hormones play a very important role in intestinal differentiation in birds and mammals (Brewer and Betz, 1982a; Subramoniam and Ramakrishnan, 1980). In young rats, THs also increase microvillus membrane formation, enhancing closure of the intestinal barrier to penetration of immunoglobulins (Jacobowitz et al., 1987). In tissue culture studies of duodenum, THs increase goblet cell differentiation, epithelial cell height and the rate of microvillar growth when compared to intact tissues (Black, 1978; Black and Moog, 1978). Use of the thyroid inhibitor, thiourea, caused retardation of villus elongation and reduced epithelial cell height. Tissue changed to a medium containing T4 caused day 14 cultured duodenum to increase epithelial cell height, stimulate microvillar growth within 24-48 hours, and increased alkaline phosphatase and maltase enzymes. The T4-treated pieces of day 14 intestinal tissue were comparable to 20 and 22 day incubated tissue of controls (Black, 1978). Intestinal epithelial cell development, intestinal alkaline phosphatase activity and sugar absorption from the gut develop in concert in relation to time in chick embryos.
(Betz and Goldberg, 1982; Black, 1988; Black and Moog, 1978; Brewer and Betz, 1979, 1982a, 1982b).

It has been suggested that T3 stimulates D-glucose uptake by intestine by stimulation of a low-affinity glucose transport system (Kimmich and Randles, 1976; Prager et al., 1990). Hyperthyroidism has been associated with increased glucose uptake in the intestine, which has also been shown in experiments in healthy humans administered T3 (Bratusch-Marrain et al., 1984).
Alkaline phosphatase in intestinal development

Alkaline phosphatase has been used as a marker indicative of cell differentiation in many tissues, including avian embryonic intestine. Since T₃ accelerates intestinal differentiation, many studies have examined the induction of alkaline phosphatase in chick intestine (Black and Moog, 1978; Brewer and Betz, 1979; Mallon and Betz, 1982). In general in vertebrates, alkaline phosphatase plays varied roles including those of transport across membranes, and phosphate transport in intestine. It acts as a barrier in the absorption of some electrolytes from the gut, in the case of salt water eels (McComb et al., 1979). The presence of alkaline phosphatase in the proximal convoluted tubule of the kidney, the intestinal microvilli, and the follicular epithelium of the ovary and placenta, suggests that it plays an important role in the active transport of substances across membranes. There is also some correlation between intestinal alkaline phosphatase activity and phosphorus absorption in the intestine. Alkaline phosphatase has different functions. It hydrolyzes phosphate esters situated at the cell surfaces, thereby helping in the transport of orthophosphates and organic residues into the cell (McComb et al., 1979). Alkaline phosphatases transport phosphates from one alcohol group to another; thus, the transferase activity serves a physiological function. Alkaline phosphatase has a high affinity for inorganic orthophosphates, therefore it is possible that the enzyme-phosphate complex oscillates rapidly between intracellular and extracellular media as it transports phosphate (McComb et al., 1979).
Genetic selection for high and low body weight in chickens

Manipulations to maximize growth rate and efficiency of feed utilization in chickens have been used to optimize their production efficiency. The lines selected for body weight are derived from a common White Plymouth Rock population. The high weight line shows more efficient feed conversion to body mass than the low weight line (Coleman and Siegel, 1966; Lepore et al., 1963; Nir et al., 1987). Associated with differences in weight between the lines, there are differences in growth rate, food consumption, lipid metabolism, and reproductive traits (Calabotta et al., 1983; Siegel, 1984; Siegel and Dunnington, 1985). There are differences in plasma hormone levels, such as growth hormone and thyroid hormones (until hatching). Plasma growth hormone is lower in the low weight line of chickens (Harvey et al., 1979; Scanic et al., 1980). Plasma T3 and T4 levels do not differ in the adults of HW line, LW line or the F1 cross (McNabb et al., 1991). The high weight adult birds have significantly higher hepatic 5'D than adults in the low weight line. Liver is one of the major contributors to peripheral T3 (Engler and Burger, 1984) and, since liver size is proportional to body weight, it has been suggested that there is higher T3 production potential in adults of the high weight line (McNabb et al., 1991).
MATERIALS & METHODS

Animals

The chickens used in this experiment were from lines of White Plymouth Rocks subjected to individual phenotypic selection for body weight at 56 days of age, for 32 generations (Dunnington and Siegel, 1985). The HW and LW lines show a 6-fold difference in body weight at the selection age.

Tissue collection and handling

Tissue samples for validation and characterization of 5'D activity were collected from adult males at 60 weeks of age. Samples for the perinatal study were obtained from late day 18 embryos through day 21, at four stages: prior to internal pipping into the air cell (NP), embryos pipped into the air cell (AC), embryos pipped through the shell (TS), chicks within 24 hrs of hatch (H).

Embryonic blood was collected into heparinized microhematocrit tubes from chorioallantoic veins of carefully opened eggs immediately after decapitation. Blood samples were centrifuged and the plasma was stored frozen until analysis for plasma T3 and T4. After blood collection, intestinal tissue (from the beginning of the duodenum to the end of the ileum, not including the ceca, the pancreas was discarded) was removed rapidly, cut open longitudinally, rinsed in saline to remove intestinal contents, blotted dry, weighed to the nearest 0.01 g, minced, divided into two portions and frozen in small cryovials in liquid nitrogen. Intestinal tissue from adults was handled in the same way, except that the entire intestine was minced, mixed thoroughly, and then the required amount was removed, weighed and stored in cryovials.
Minced intestinal tissue was thawed with ice-cold buffer and homogenized with a Brinkmann Polytron with a generator for 30 sec in a glass tube, held in ice water. Tissues for both the 5'D and ALP assays were handled in the same way except that tissues for the ALP assay were prepared and stored in 0.9% NaCl as whole homogenate at -20°C until time for assay. Tissues used for the 5'D assay were prepared and stored in 50 mM morpholinopropane sulfonic acid (MOPS) with 1 mM EDTA at pH 7.4. Homogenate was centrifuged at 12,100 g at 4°C for 2 min. Supernatant from two washes, with a total volume of six times the original tissue weight, yielded the final post-mitochondrial fraction (PMF). Post-mitochondrial fraction was frozen in 100-500 µl aliquots in liquid nitrogen, and stored at -20°C until further use.

Analytical Techniques

5'D assay: In vitro activity of intestinal 5'D was measured by the general method used by McNabb et al., (1991) for 5'D assays on adult liver tissue from these lines. The conditions stated below were validated and used for the comparison of intestinal 5'D in the adults of the two lines. The incubation times and PMF concentrations used in the assays for the developmental stages were based on validation studies for each stage and are presented in Table 2 in Results.

5'deiodinase activity was measured in vitro as the rate of release of radioactive iodine from high-specific activity 125-I labeled reverse-T3 (rT3) (New England Nuclear Corp, Boston, MA; 750 - 1,200 µCi/µg), by intestinal tissue PMF. The conditions used for experimental comparisons were those shown by validation studies to result in linearity of enzyme activity with incubation time and proportionality of enzyme activity with enzyme concentration.
Assay tubes were held on ice during the addition of assay components. Total assay volume of 160 µl consisted of 50 µl PMF (0.4 mg tissue), 40 µl dithiothreitol (DTT; as cofactor at 20 mM final concentration), 20 µl of MOPS buffer, 40 µl unlabeled rT3 (4 µM final concentration), 10 µl high specific activity 125I-rT3 (approximately 100,000 cpm). After addition of all components to each tube, the tubes were vortexed and incubated in a 37.5°C shaking waterbath for 45 minutes. The reaction was stopped and protein was precipitated by addition of 40 µl ice-cold 4% BSA in MOPS buffer followed by 300 µl ice-cold 20% TCA. Tubes were centrifuged at 4000 g at -10°C for 30 mins. Blanks, for which the reaction was stopped immediately (i.e. no incubation), were used to correct for presence of non-hormonal 125-I background in original labeled hormone preparation. Blanks without tissue, that were incubated, indicated that incubation did not increase amount of 125-I background.

After protein precipitation, a 400 µl aliquot of resulting supernatant was loaded onto Biorad "Dowex" AG 50W-X8(H+) ion exchange columns (Biorad Laboratories, Richmond, CA) and eluted by two 5 ml additions of glacial acetic acid. Each 5 ml fraction was collected separately and counted on a Tram Analytic gamma counter for 5 min.

ALP assay: Alkaline phosphatase was measured in vitro in intestinal homogenates using Sigma Diagnostics Alkaline Phosphatase Components, based on the procedure described by Bowers and McComb (1966). Alkaline phosphatase degrades p-nitrophenyl phosphate, which results in development of color when phosphate is removed and p-nitrophenol is formed. P-nitrophenol was measured as absorbance at 405 nm. The total assay volume of 1.02 ml contained of 20 µl of diluted homogenate. Two modifications of the Sigma procedure were used: the reaction was at room temperature (22°C) and reaction time used was 20 min.
Thyroid hormone measurements: Plasma T4 and T3 were determined with a double antibody radioimmunoassay (RIA: method of McNabb and Hughes, 1983) using standards prepared in hormone-free chicken plasma. Primary antibodies from Endocrine Sciences (Tarzana, CA), carrier immunoglobulin from Antibodies Incorporated (Davis CA), and second antibody produced in sheep by Dr. Larry Cogburn (Univ. of Delaware), were used. Assay volumes were 12.5 µl for T4 and 25 µl for T3. Precision tests of the assay indicated that coefficient of variation was 1.3% for T4 (N=10) and 2.3% for T3 (N=10). Accuracy was 97.1% for T4 and 100.5% for T3, against a euthyroid control serum (Ortho Diagnostics Inc., Raritan, NJ). Lower limits of assay sensitivity were 1.25 ng/ml for T4 and 0.125 ng/ml for T3.

Protein Determination: Protein concentrations of PMF were determined using Biorad protein assay (Biorad Laboratories, Richmond, CA) modified to accommodate the small sample volumes available. The standard curve was 0 to 1.4 µg BSA/µl in MOPS buffer, standard sample volume was 40 µl, dye reagent volume was 2 ml. Other details of the assay were carried out according to the directions of the manufacturer. All standards and samples were assayed in duplicate. Coefficient of variation on 10 aliquots of a pooled sample was 0.25%.

Experimental design

Enzyme assay validation: To establish initial velocity conditions two types of experiments were performed. First the range over which activity was proportional to tissue (i.e. enzyme concentration) was determined using appropriate PMF concentrations. Second, the range over which activity was proportional to incubation time for different concentrations of the PMF was determined. These assay validations were carried out for both 5'deiodinase and alkaline phosphatase enzymes.
Characterization of rT3 5'D activity: In the characterization assays, conditions used were those validated for adult intestine (see standard assay described above), but the following were tested. Apparent $K_m$ and $V_{max}$ were determined by using a range of substrate concentrations (0.05, 0.10, 0.20, 0.40, 0.80, 1.0, 2.0, 4.0 μM unlabeled rT3). Cofactor dependency was determined using a range of DTT concentrations (1, 5, 10, 20, 30, 50 mM). Effects of pH were determined over a range of half pH intervals from 5.5 to 9.0. Effects of inhibitors on 5'D activity were determined with 1.0 mM PTU and 0.5-2.5 mM IOP in the reaction mixture.

Statistical analyses: Regression analyses for kinetic data were made using an unweighted least squares procedure. From kinetic studies, $K_m$, $V_{max}$ and their standard errors were calculated by the statistical method of Cornish-Bowden (1976). Where appropriate, data were analyzed by one way analysis of variance and Tukey's HSD. Values of $p<0.05$ were considered indicative of statistically significant differences.
RESULTS

Validation and characterization of 5'D activity in adult intestine: comparison between high and low weight lines.

Intestinal 5'D activity (as pmol of accumulated product) was proportional to tissue (enzyme) concentrations from 0.1 to 0.5 mg tissue/tube for tissue pools from both lines (N = 6 birds/line, Fig. 1a). This proportionality resulted in constant activity (pmol product/min.mg tissue) of 5'D for this range of tissue concentrations (Fig. 1b). 5'-D activity was linear with incubation times up to 90 minutes, at concentrations of 0.13 and 0.40 mg tissue/tube, for tissue pools from both lines (N = 6 birds/line, Fig. 2). In each of these experiments, 5'D activity in pooled HW tissue was higher than that in pooled LW tissue.

Characterization studies of 5'D activity showed Km values that were similar and between 0.1 and 0.8 µM in both lines (Table 1). A study of cofactor effects showed increasing 5'D activity with increasing cofactor, up to 20 mM DTT, and a slow rate of increase in activity from 20 to 50 mM DTT. 5'D activity was highest, and did not differ significantly, between pH 7.5 and 8.0 for the HW line and between pH 8.0 and 8.5 for the LW line. Activity was significantly lower at all other pH conditions tested. Intestinal 5'D activity was inhibited by >90% by 1.0 mM PTU in both lines. An IOP concentration of 1.5 mM was required for >95% inhibition in the HW line, and 2.5 mM for >93% inhibition in the LW line.

Comparison of adult intestinal tissue samples from 6 individuals from each of the two lines showed significantly (p < 0.05) higher 5'D activity in the HW line, mean 6.9 ± 0.2, than in the LW line, mean 3.7 ± 0.3.
Plasma thyroid hormones during the perinatal stages of development.

Both lines showed sequential increases in plasma T4 and T3 from the NP stage through to the TS stage (Fig. 3). The TS stage was greater than the NP stage in both lines. After peaking at the TS stage, plasma concentrations of both thyroid hormones decreased in both lines. Both plasma hormones were higher in the LW line than the HW line embryos at each stage. Plasma T4 was not significantly different at the NP and H stage (Fig. 3).

Validation of 5'D and alkaline phosphatase activity in intestine from perinatal stages.

For both alkaline phosphatase and 5'D activity a range of valid conditions was defined. Proportionality of enzyme activity with tissue concentration and linearity of activity with incubation time was determined for each of the perinatal stages for each line. Validation studies on each developmental stage indicated that the measurement of intestinal 5'D at initial velocity could be achieved with 45 minute incubation times for embryos at the NP stage and for embryos at the AC stage but 15 minute incubation times were needed to achieve maximal conditions for the older stages. This reflects higher enzyme content in older embryos. Validation studies were done at each stage in the case of alkaline phosphatase. Initial velocity conditions were achieved using higher concentrations of homogenate to get measured activity in the youngest stages; with sequential dilutions reduced concentrations of homogenate were used at later stages to prevent limiting conditions, and to maintain initial velocity conditions.
Comparison of intestinal 5'D, alkaline phosphatase activity and relative intestinal weights during the perinatal period in high and low weight lines.

5'-deiodinase activity increased significantly in both lines as they progressed through the perinatal period to the TS stage (Fig. 5a). The LW embryos showed significant increments in activity at each sequential stage and reached peak activity at the TS stage; embryos at the TS stage and H chicks did not differ in 5'D activity. The HW embryos showed much lower 5'D activity than the LW embryos, although 5'D increased in significant increments at each stage including H chicks. Alkaline phosphatase activity increased significantly between each of the stages of development in each of the lines. The LW line showed higher alkaline phosphatase activity than the HW line at all stages (Fig. 5). Relative intestinal weight (as % body weight) did not differ in the two lines except at the H stage, where the HW line showed two fold higher relative intestinal weight than the LW line (Fig. 4b).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
FIGURE LEGENDS

Fig. 1. Proportionality of intestinal rT3 5'D activity with enzyme concentration in pooled tissue samples from adult selected, high (HW) and low (LW) weight lines. (a) Accumulation of product during 45 min. incubation. (b) specific activity. Assay: 5'D activity measured as iodide (pmol I⁻) released from 4 μM rT3 as substrate by postmitochondrial fraction, 20 mM dithiothreitol, 45 min. incubations. Each data point is the mean of triplicate determinations.

Fig. 2. Linearity of intestinal rT3 5'D activity with incubation time by pooled samples from adult high and low weight selected lines of chicken. Assay: 5'D activity measured as of iodide released from 0.4 μM rT3 as substrate by postmitochondrial fraction from 0.4 mg tissue with 20 mM dithiothreitol. Each data point is the mean of triplicate determinations.

Fig. 3. Plasma thyroid hormone concentrations during the perinatal period in high (open bars) and low (shaded bars) weight lines of chickens. NP = embryos not pipped into the air cell; AC = embryos pipped into the air cell; TS = embryos pipped through the shell; H = hatchlings within the first 24 h; N = 10 for each line at each stage. Vertical lines on the bars are the ±1SE. Asterisks designate differences between the lines within a stage (p<0.05). Differences between stages within lines are described in the text.

Fig. 4. Intestinal 5'-deiodinase and relative intestinal weight on days 19 through 21 of incubation in high (open bars) and low (shaded bars) weight lines of chickens. (a)
Intestinal 5'D activity as 125-I release from labeled rT3 during the perinatal period in high and low weight lines of chickens. (b) relative intestinal weight (c) total intestinal 5'-deiodinase activity per g body weight. NP = embryos not pipped into the air cell; AC = embryos pipped into the air cell; TS = embryos pipped through the shell; H = hatchlings within the first 24 h; N = 10 for each line at each stage. Vertical lines on the bars are the ±1SE. Asterisks designate differences between the lines within a stage (p<0.05).

Fig. 5. Intestinal alkaline phosphatase activity during the perinatal stages of development in high (open bars) and low (shaded bars) weight lines of chickens. N=10. NP = embryos that had not pipped into the shell; AC = embryos that had pipped into the air cell; TS = embryos that pipped through the shell; H = 1d post hatch chicks. Vertical lines on the bars are the ±1SE. Asterisks designate differences between the lines within a stage (p<0.05).
**TABLE 1: Michaelis-Menten Values for Intestinal 5’D Activity in Pooled Samples**

From Adults of High and Low Weight-selected Lines of Chickens

<table>
<thead>
<tr>
<th>Tissue Pool</th>
<th>Km (µM)</th>
<th>Vmax (pmol I/min mg PMF protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HW</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.76±/0.21</td>
<td>1.85±/0.33</td>
</tr>
<tr>
<td>2</td>
<td>0.37±/0.33</td>
<td>2.62±/0.59</td>
</tr>
<tr>
<td>˚X</td>
<td>0.56</td>
<td>2.23</td>
</tr>
<tr>
<td><strong>LW</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.71±/0.31</td>
<td>2.23±/0.63</td>
</tr>
<tr>
<td>2</td>
<td>0.17±/0.45</td>
<td>1.41±/0.31</td>
</tr>
<tr>
<td>˚X</td>
<td>0.44</td>
<td>1.83</td>
</tr>
</tbody>
</table>

5’D activity was measured as iodide (I⁻) released from reverse T3 substrate at 4µM concentration, by postmitochondrial fraction with 20 mM dithiothreitol. Tissue pools with the same number for HW and LW indicate pooled samples collected and assayed at the same time. Values are the mean ± SE.
DISCUSSION

Assay Validation and characterization of 5'D activity in adult intestine.

To my knowledge, no previous studies have examined 5'D in avian intestinal tissue. Therefore, validation and characterization of 5'D activity were conducted in adult chicken intestines. To make accurate quantitative measurements of enzyme activity it was necessary that enzyme assays measure initial velocity conditions. This ensured that activity measurements were not underestimates resulting from enzyme inhibition, enzyme inactivation or substrate limitation.

Previously, many avian studies of 5'D activity have used T4 as a substrate without validating assay conditions. When T4 is used as a substrate, multiple deiodination reactions may occur, with T4 being deiodinated at both the inner (5) and outer (5') positions, followed by further deiodination of the products (Galton and Hiebert, 1987; Leonard and Visser, 1986). To overcome this problem, I used rT3 as a substrate to measure 5'D activity because it gives a single reaction system in which rT3 is degraded only to T2 and I- (see discussion in Freeman and McNabb, 1991 and McNabb and Freeman, 1990).

Enzyme activity in intestinal tissue is approximately half that of hepatic 5'D activity. These low intestinal activities cannot be compared to Chopra's study of intestinal 5'D activity in rats (Chopra, 1977) because the latter study used T4 as a substrate, and employed conditions unlikely to have achieved initial velocities. Chopra found extremely low activity in intestinal tissue when compared to liver and kidney. In comparison to our study of chicken intestine, recent studies on adult rat intestinal tissue showed barely detectable amounts of 5'D activity (Galton et al., 1991).
This study shows that intestinal 5'D in adult chickens has characteristics similar to avian and mammalian hepatic 5'D. The kinetic studies indicate that chicken intestinal 5'D activity has a Km in the low micromolar range, similar to most measurements of hepatic 5'D with rT3 as substrate (refer to Table 1 and 2).

The requirements of intestinal 5'D in these lines for thiol cofactor, with rT3 as substrate, are similar to those in mammals and other bird species (chicken embryos: Galton and Hiebert, 1987; quail: Freeman and McNabb, 1991; doves: Rieman and McNabb, 1990; rats: Galton et. al., 1991; Leonard and Rosenberg, 1980).

The two lines of chickens had overlapping plateaux of optimal pH values (HW line - pH 7.5 to 8.0 and LW line - pH 8.0 to 8.5). These values are comparable to those in other avian and mammalian studies (optimum for rat liver - pH 8.1, Visser et al., 1979; quail liver pH 8.0 - 8.5, Freeman and McNabb 1991; dove liver pH 8.04 , Rieman and McNabb, 1990).

The studies of PTU inhibition at 1.0 µM showed a similar sensitivity of intestinal 5'D to that of rat liver and kidney (Leonard and Visser, 1986), and quail, dove and chicken liver (Freeman and McNabb 1991; Rieman and McNabb 1990; McNabb et al., 1991). This inhibition by PTU suggests that we are measuring a Type I activity. The LW showed less sensitivity to IOP than the HW line. Inhibition of >90% of intestinal 5'D required a higher concentration of IOP (1.5 mM-HW; 2.5 mM-LW) than for 5'D from chicken liver, which was inhibited at 1.0 mM IOP. Inhibition by IOP of >90% verified that the activity being measured was that of 5'D enzyme.

Results of this study indicate that a type I deiodinase in intestinal tissue was measured. The type I deiodinase has been characterized with a Km value ~ 1 µM. This pathway is
responsible for the greatest amount of deiodination by both inner and outer ring deiodinations. The physiological role of type I deiodinase is to provide T3 to peripheral tissues by deiodination carried out by organs such as liver. In studies by Galton, rat intestine showed a Km value in the low micromolar range comparable to the Km value in our study. That study also was comparable to our study in the use of thiol cofactor, DTT, and also showed inhibition with IOP and PTU (Galton et al., 1991). In contrast to the type I intestinal enzyme in chickens and mammals, amphibian intestine had a low Km, type II 5'D activity (Galton, 1988a).

**Comparison of intestinal 5'D activity in adults of high and low weight lines.**

When we compared adult intestinal 5'D activity from individual birds from each line, using the validated conditions, we found intestinal 5'D activity was approximately two fold higher in the HW line than the in LW line (Fig. 4). This line difference in intestinal 5'D is similar to that shown by previous measurements of adult hepatic 5'D in the two lines (HW line 160% that of LW line) (McNabb et al., 1991).

**Perinatal studies:**

Genetic studies done with these lines and their crosses have demonstrated that hatching, as well as movement into each of the perinatal stages, is earlier in the LW line than the HW line (Dunnington et al., 1992). Concentrations of plasma thyroid hormones measured during the perinatal stages of development were significantly higher in the LW line than the HW line. Plasma thyroid hormone patterns showed a gradual increase from stage to stage, with peak values in embryos at the TS stage. Results from this study are consistent with other studies done in quail and chickens, with a peak in plasma thyroid hormone values just prior to hatching (Freeman and McNabb, 1991; McNabb et al., in manuscript).
Higher thyroid hormones have been found to advance hatching. Our results also reflect this, since the LW line show higher concentrations of thyroid hormones and advanced hatching times than HW line. Increased T3 (the active thyroid hormone) availability in the LW line during the perinatal stages of development may be advancing the hatching time of the LW line (Dunnington et al., 1992; McNabb et al., in manuscript). Previous studies show that addition of T4 or T3 to eggs accelerated the rate of hatching (Christensen, 1985). It was then suggested that T3 played a role in the earlier hatching times of the chicks, as treatment of eggs with PTU or IOP (both act as inhibitors) delayed hatching.

**Intestinal 5'D, alkaline phosphatase activity and relative intestinal weights during the perinatal period in high and low weight lines.**

Intestinal 5'D in both lines of chickens showed increasing capability for T3 production during the perinatal stages of development. Intestinal 5'D increased almost 5 fold from embryos of the NP stage to H chicks. The HW lines showed increasing levels of 5'D activity between each stage of development. The LW line reached peak activity at the TS and H stage. Thus, both lines showed a similar pattern with increasing levels of 5'D from the NP to the TS stage. Each stage of development in both lines is associated with a quantitative increment of alkaline phosphatase (which is indicative of tissue differentiation) at that stage. Increasing values of alkaline phosphatase activity at each stage directly reflected the amount of intestinal development, and are consistent with T3 inducing intestinal maturation.

Intestinal alkaline phosphatase was measured to see if intestinal maturation in the LW line was advanced and consistent with earlier hatching times of the LW line. This study of alkaline phosphatase, in the same embryos that 5'D activity was measured, found that alkaline phosphatase activity was higher in the LW line than in the HW line. This result suggests that internal mauration is more rapid in the LW line, consistent with earlier
potential for food assimilation after earlier hatching (Dunnington et al., 1992)

During the perinatal stages of development comparison of the two lines showed the opposite pattern of 5'D activity in embryos to that in adults (i.e. the LW line embryos have shown higher intestinal 5'D activity than the HW line embryos, whereas in adults this pattern was reversed). The perinatal period of the LW line was at a more advanced stage shown by the earlier hatching times. The shift in development occurred soon after hatching. The HW line showed immediate posthatch growth, which was demonstrated in a large increase in the measurements of relative intestinal weights in the hatchlings. This increase was not found in the hatchlings of the LW line. This indicates that the whole intestinal 5'D potential per unit body weight, in the HW line chicks, is much greater than the LW line. This picture is consistent with other studies on these lines where chicks of the HW line showed greater relative intestine growth and feed efficiency than those of the LW line. Chicks from the HW line also showed higher levels of pancreatic digestive enzymes. These studies found that the HW line advanced at a much faster rate through the early posthatch period of their life than the LW line (O'Sullivan et al., 1992a and 1992b).

The relationship between T3 and intestinal development has been well established. Previous work indicates that T3 induced intestinal digestive enzymes (e.g. maltase) and enhanced glucose transport across the intestinal wall (Black, 1988; Black and Moog, 1978). Studies which examined the effects of thyroid hormones on tissue development, including studies on avian embryonic intestine (Black and Moog, 1978; Mallon and Betz, 1982), used alkaline phosphatase as a marker indicative of cell differentiation. In addition to the transport of phosphate molecules, it is suggested that alkaline phosphatase may participate in the sugar transport mechanisms (Mallon and Betz, 1982). Intestinal epithelial cell development, duodenal alkaline phosphatase activity, and sugar absorption develop concurrently in chick embryos (Black, 1978; Black and Moog, 1978; Brewer and Betz,
1979, 1982a, 1982b). Thus, thyroid hormones play a role to stimulate these developmental changes. Other studies found T4 and T3, introduced into eggs of developing chick embryos or intestinal tissue grafts cultured in medium, accelerated intestinal cell development (Black, 1978; Black and Moog, 1978; Brewer and Betz, 1979, 1982a; Mailon and Betz, 1982). Further, inhibition of thyroid function caused retardation of epithelial cell height and normal development was restored by injections of thyroxine (Moog, 1961). Thyroxine also was found to promote glycogen and glucose metabolism in cultured embryonic chick intestine (Black, 1988). These thyroxine effects appear to be due to the formation of T3 by deiodination, with T3 stimulating sugar transport just prior to hatching (Black, 1988; Holdsworth and Wilson, 1967). In other studies, T3 was found to stimulate D-glucose uptake by cultured small intestine of embryonic chick, presumably by stimulation of a low-affinity transport system (Prager et al., 1990).

The perinatal peak in plasma T3 contributes to hatching and the many developmental changes occurring in the embryo. Results from this study, of increasing 5'D activity, demonstrate the potential for increasing conversion of T4 to T3 in the intestine. It is also possible that the increasing levels of plasma T3, measured in this study, contribute to the T3 pool in the intestine to stimulate intestinal maturation. Since T3 is the metabolically active hormone in birds, it presumably has a role in triggering the increasing ALP activity (indicative of cell differentiation), and thus intestinal maturation of the developing embryo. One indication of intestinal maturation is stimulation in sugar transport mechanisms by T3 near hatching (Black 1988). The 5'D measurements demonstrate that the intestinal tissue itself produces some of the T3 involved in the intestinal cell maturation. Higher T3 production in the LW line, which hatches earlier than the HW line (Dunnington et al., 1992) may play a role in earlier maturation of intestine of the LW line which could permit food assimilation soon after the chick hatches. The LW line birds have been found to be anorexic and have a very low feed intake at hatching and as adults (O'Sullivan et al.,
1992a; Zelenka et al., 1988). This has been found to be a behavioral modification since intermingling of the lines in cages caused a higher percentage of the LW line birds to eat and drink than those kept separately (Noble et al., 1992). The earlier maturation of the intestine of the LW line suggests that this could be a physiological change in anticipation of feeding soon after hatching. The behavioral and physiological changes in the LW line are independent of one another.
LITERATURE CITED

Allen, B. M. 1932. The response of Bufo larvae to different concentrations of thyroxine. Anat. Rec. 54(1), 45-64.


Harvey, S., C. G. Scanes, A. Chadwick, and N. J. Bolton. 1979. Growth hormone and


McNabb, F. M. A., E. A. Dunnington, P. B. Siegel, and, S. Suvarna. (In manuscript). Perinatal thyroid hormones and hepatic 5′deiodinase in relation to hatching time in weight selected lines of chickens.


Schwartz, H. L., 1983. Effect of thyroid hormone on growth and development. In: Molecular basis of thyroid hormone action. eds., J. H. Oppenheimer, and H. H. Samuels,


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Suvarna, S., F. M. A. McNabb, E. A. Dunnington, and P. B. Siegel. Intestinal
5’deiodinase activity of developing and adult chickens selected for high and low body

F. M. A. McNabb, E. A. Dunnington, P. B. Siegel, and S. Suvarna. Perinatal Thyroid
Hormones and Hepatic 5’deiodinase in relation to Hatching Time in Weight Selected Lines

PRESENTATION AT SCIENTIFIC MEETINGS


