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by

Bruce Leslie Carr

A Dissertation Submitted to the Faculty of the COMMITTEE ON ANIMAL PHYSIOLOGY (GRADUATE) In Partial Fulfillment of the Requirements For the Degree of DOCTOR OF PHILOSOPHY In the Graduate College THE UNIVERSITY OF ARIZONA

1 9 8 1
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Bruce Leslie Carr entitled **Effects of Thyrotropin Releasing Hormone and Environmental Temperature on the Hypophysial-Thyroid Axis of Hypothyroid, Euthyroid and Castrated White Leghorn Chickens**

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Fig. 3.4.1  

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ABSTRACT

Cyclic AMP-dependent protein kinase (cAMP-PK) is an important mediator of hormone action. Its activity ratio is an accurate indicator of cellular activity under various experimental conditions including: (1) age and sex, (2) hormone administration and (3) temperature and photoperiod.

Pituitary activity in unstimulated birds is not altered by age, but thyroid activity is much higher in old birds than in young animals. Thyrotropin releasing hormone (TRH) increases pituitary, thyroid and liver activity of prepubescent chickens, but has no effect on aged males and increases only thyroid and liver activities in aged females, suggesting a reduction in pituitary-thyroid function with advancing age.

In prepubertal females, TRH increases pituitary and thyroid cAMP-PK activity, plasma $T_3$ and $T_4$ levels and liver $T_4$ monodeiodination. Thyroid activity reaches maximum activity before the pituitary, while plasma $T_4$ and liver $T_4$ monodeiodinating activity reach their highest levels 20 minutes before plasma $T_3$. These findings suggest that fluctuations in liver $T_4$ 5' monodeiodinating activity might be responsible for the cyclic response of plasma $T_3$ and $T_4$.

Castrated cockerels have larger pituitaries than untreated birds, but contain the same amount of DNA. Methimazole-fed cockerels have pituitaries significantly smaller than controls, while castrated cockerels fed methimazole have pituitaries the same size as untreated
birds. Pituitary DNA is less than controls in both groups of methimazole-fed birds. These results are considered to be due to a change in the thyrotroph population, without an increase in total cell numbers, and may indicate a transformation of basophils. Pituitary cAMP-PK activity during cold stress substantiates this conclusion.

Thyroid glands of castrated and untreated cockerels are similar in size, histological appearance and DNA content; however, cAMP-PK activity is much greater in the castrated birds. Methimazole-fed cockerels have enlarged thyroid glands, elevated cAMP-PK activity, increased DNA and cellular hypertrophy; however, these effects may be mitigated by castration.

Seven days after removal of testosterone supplements, photo-stimulated castrates have a higher thyroid cAMP-PK activity ratio than short day castrates; however, both groups are elevated above control, suggesting that long photoperiods enhance the stimulatory effects of castration on thyroid activity. Pituitary activity is elevated in long and short day birds seven days after removal of testosterone, but remains high only in short day castrates. Therefore, a reduction in the sensitivity of the hypothalamic-pituitary axis to testosterone may occur only in long day cockerels.
INTRODUCTION

The Hypothalamic-Hypophysial-Thyroid Axis

The term "hypothalamic-hypophysial-thyroid axis" is generally used to describe the relationships that exist between the hypothalamus, adenohypophysis and the thyroid. The ability to maintain relatively constant plasma levels of thyroid and thyroid stimulating hormone is dependent upon this relationship. Thyrotropin releasing hormone (TRH) from the hypothalamus increases the plasma levels of thyroid stimulating hormone (TSH) and the resulting increase in thyroid hormone decreases plasma TSH levels by a negative feedback mechanism (1).

Thyrotropin releasing hormone is thought to be produced by neurons of the rostral-basal hypothalamus (2, 3, 4) and released by their axons in the median eminence. These axons are in close association with capillary loops of the hypothalamic-hypophysial portal vessels (5). The portal vessels transport TRH to the sinusoids of the adenohypophysis where TRH binds to thyrotroph cell membrane receptors, thus stimulating adenylate cyclase activity. Adenylate cyclase in turn generates 3', 5' adenosine monophosphate (abbreviated as either cAMP or cyclic AMP), which eventually leads to the release of TSH into the systemic circulation. In a similar manner TSH binds to membrane receptors on thyroid follicular cells and results in an increased release of thyroxine ($T_4$) and/or triiodothyronine ($T_3$).
Low ambient temperature elevates pituitary TSH secretion in mammals by increasing hypothalamic secretion of TRH (6, 7). Noradrenergic neurons from hypothalamic temperature receptors or higher brain centers may be responsible for the increased TRH secretion (8). However, this system is probably not essential for thermoregulation in birds (9). It is doubtful if the avian hypothalamus has a thermo-receptive role (9), and there appears to be considerable species variation in response to hypothalamic heating or cooling. The spinal cord and skin are the primary sources of information for regulating hypothalamic function in birds (10). Dopamine, serotonin and somatostatin may enhance TSH release in mammals (8), but their effects on TSH secretion in birds are not known.

The concept of a hypothalamic-hypophysial-thyroid axis is herein extended to include the liver, a major target tissue of thyroid hormone and the major site of thyroxine monodeiodination (11, 12, 13). Since birds lack a thyroxine binding globulin (14), thyroxine and triiodothyronine are bound to albumins and prealbumins produced by the liver. The binding affinity of albumin for $T_4$ is greater than for $T_3$ (15), and prealbumins bind $T_3$ in the pigeon but not in any other vertebrate species reported (16). The binding of $T_4$ to avian albumins is not very strong compared to that in man, and as a result there is more free $T_4$ in avian than in human serum (16). The deiodination of plasma $T_4$ by the liver is the major source of circulating $T_3$ in mammals (17, 18, 19) and birds (13, 20), although avian serum $T_3$ levels are in a range that would be considered hyperthyroid in mammals. The liver is
also involved in the degradation of thyroid hormones by formation of \( T_3 \) and \( T_4 \) glucuronide and sulphate conjugates, resulting in their subsequent excretion in the bile and urine (21).

**Cyclic AMP-Dependent Protein Kinases in Hormone Action**

Proposal of the second messenger concept by Sutherland (22) stimulated considerable investigation on cyclic nucleotides and their role in the mechanism of action of peptide hormones. Sutherland maintained that the hormone serves as the first messenger and that its effects are translated intracellularly by the second messenger, cyclic AMP. Cyclic AMP regulates the enzymes of intracellular metabolic pathways, thus controlling cellular activity. Walsh (23) later established a dependency of a portion of the cytoplasmic protein kinase on intracellular cAMP. Cyclic AMP forms a complex with the protein kinase molecule allowing subsequent molecular events to occur. It is now apparent that several different hormones increase the activity of intracellular cAMP in a variety of tissues (24). Three hypothalamic peptides and several pituitary hormones act by way of the cAMP/cAMP-dependent protein kinase mechanism (25, 26, 27). The binding of cAMP to the regulatory subunit of cAMP-dependent protein kinase appears to be a unique combination for cAMP and an intracellular protein suggesting that all of the actions of cAMP are mediated by the phosphorylation of proteins.

Thyrotropin releasing hormone stimulation of a pituitary thyrotroph is illustrated in Figure 1, although the cellular events are
Figure 1. Diagrammatic summary of the mechanism of action of thyrotropin releasing hormone and thyroid hormones on the pituitary thyrotroph.
probably applicable to any target cell. In addition to TRH, all polypeptide hormones stimulate their target gland by first engaging a functionally specific receptor on the outer membrane surface of the target cell.

The binding of a protein hormone to its membrane receptor initiates the activation of adenylate cyclase which in turn stimulates the intracellular conversion of adenosine triphosphate (ATP) to cAMP. The holoenzyme \((R_2C_2)\) of cAMP-dependent protein kinase contains two subunits; a regulatory subunit \((R)\), which binds the intracellularly formed cAMP, and a catalytic subunit \((C)\), which contains the active site. In the presence of saturating levels of cAMP the holoenzyme binds two molecules of cAMP per \(R_2C_2\) unit and dissociates to give one regulatory subunit dimer \((R_2)\) and two catalytic subunit monomers \((C)\) according to the equation:

\[
R_2C_2 \text{ (inactive)} + 2 \text{cAMP} \rightleftharpoons R_2 \text{(cAMP)}_2 + 2 \text{C (active)}
\]

In this reaction, there is a constant dissociation of the holoenzyme and reassociation of the subunits to equilibrium.

Purification of the holoenzymes of cAMP-dependent protein kinase by chromatography on DEAE cellulose has identified two forms of the enzyme, type I and type II. The proportions of type I and II forms differ widely between tissues of a single species and for a specific tissue between species. Current evidence suggests that the catalytic subunits of type I and II protein kinase are identical but the regulatory subunits are distinctly different and may be separated by their
difference in net charge. Nimmo (28) summarizes many of the differences between the two forms; however, two differences of critical importance are their ability to bind cAMP and their properties of association and dissociation.

The active catalytic subunit catalyzes the transfer of the terminal phosphate of ATP to the serine and threonine residues in a variety of enzymes (29, 30), histones (31, 32), plasma membranes (27) and free and bound ribosomes (33, 34, 35, 36). The factors responsible for the specificity of the catalytic subunit are not known (37). However, several investigators (37, 38, 39) believe that the subcellular localization of the holoenzyme or the catalytic subunit may play a role in determining the selective activity of the two forms of the enzyme in preferentially phosphorylating specific substrates (40).

Several workers believe cAMP is capable of regulating transcription of nuclear DNA (41, 42, 43) and therefore the key enzymes in biosynthetic pathways leading to protein synthesis. Perhaps the most convincing evidence for a cAMP mediation of transcription is its ability to induce ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine biosynthesis (44). Cyclic AMP analogues and phosphodiesterase inhibitors can mimic the actions of many trophic hormones by initiating ODC activity (45, 46, 47). A transcriptional requirement for ODC activity is suggested, since administration of inhibitors of RNA and protein synthesis block any induced increase in ODC activity (46, 48) but not in cAMP-dependent protein kinase (49). There is also a one to
four hour delay between the maximum activation of cAMP-dependent protein kinase and maximum ODC activity (46, 48, 49, 50) which might be the time required for transcription to occur. Therefore, phosphorylation of the gene for ornithine decarboxylase mRNA synthesis appears to be promoted by a rise in cAMP-dependent protein kinase activity (50).

Ornithine decarboxylase is involved in the decarboxylation of ornithine to yield putrescine (Figure 1). Subsequently, S-adenosyl-L-methionine decarboxylase (SAMD) catalyzes the decarboxylation of S-adenosyl-L-methionine and the transfer of a propylamine group \((\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_3^+)\) from the decarboxylated S-adenosyl-L-methionine to putrescine, forming spermidine. An additional propylamine group from decarboxylated S-adenosyl-L-methionine is transferred to spermidine to form spermine.

Elevated spermidine levels increase RNA production in chick embryos (51), rat brains (52), sea urchin embryos (53) and larval frogs (54). A dramatic correlation occurs between accumulation of spermidine and ribosomal ribonucleic acid (rRNA) in the rat mammary gland during lactation and involution (55). When the rat mammary actively secretes, there is a concurrent increase in the intracellular concentrations of spermidine and rRNA. Conversely, during involution the concentrations of spermidine and rRNA decrease synchronously. It is believed that the polyamines act to increase the activity of RNA polymerase which initiates rRNA synthesis (56, 57). The increased nuclear production of rRNA involves a series of complex steps which eventually lead to the increased formation of ribosomes in the cytoplasm. The ribosomes aggregate on the
endoplasmic reticulum and allow for increased protein synthesis. The synthesized protein (TSH in Figure 1) is packaged within the Golgi apparatus and released as secretory granules into the cytoplasm.

In addition to its role in protein synthesis, cAMP-dependent protein kinase is also involved in phosphorylation of cell and secretory granule membrane proteins (27) which together appear to permit release of the hormone from the cell. Lemay (27) suggests that the hormone is secreted by the fusion of the vesicular membrane enclosing the hormonal granules with the cell membrane and subsequent exocytotic release of the granules. Labrie (26) indicates that phosphorylation of membrane proteins initiates alterations in the rate of the membrane fusion-fission process which subsequently modify the rate of exocytosis and hormone release. In this way, the induction of cAMP-dependent protein kinase by a trophic hormone will stimulate both hormonal synthesis and release (58).

Thyroid hormones are known to be antagonistic to TRH activation of the pituitary thyrotroph (59), but the mechanism by which they inhibit TSH synthesis and release is not completely understood. It is suggested that thyroxine and triiodothyronine increase intracellular phosphodiesterase (60), an enzyme that hydrolyzes cAMP into inactive 5'AMP, resulting in a lower cAMP concentration and consequently decreased cAMP-dependent protein kinase activity. Unfortunately, a direct connection between intracellular concentrations of thyroid hormones and phosphodiesterase has not been established. Therefore, in Figure 1 the inhibitory action of T4 on cAMP is marked by an unknown
"U" substance (59). The method by which the cell produces the "U" factor is not known, but it could be a result of increased $T_3$ binding to nuclear receptors possibly located on the DNA molecule (61, 62, 63, 64).

Thyroxine and triiodothyronine are capable of penetrating the cell membrane of the pituitary thyrotroph and $T_3$ may be found in either the cytoplasm or the nucleus (65). Thyroxine may also be found in the nucleus, but it is usually converted intracellularly to $T_4$, and the $T_3$ subsequently enters the nucleus. The nuclear receptors in the liver and kidney have a 5-10 fold higher affinity for $T_3$ than $T_4$ (66, 67, 68), therefore suggesting that $T_3$ may be the physiologically active form of the hormone.

Larsen and Silva (69, 70, 71, 72, 73) suggest that high concentrations of both plasma $T_3$ and $T_4$ are required to inhibit pituitary TSH secretion. Two hours after injection of 70 ng $T_3$/100 g body weight into hypothyroid rats there is a three-fold increase in nuclear $T_3$ and a 50 percent decrease in plasma TSH. However, during the subsequent five-hour period nuclear $T_3$ concentration decreases and plasma TSH increases until pretreatment levels are reinstated. Plasma $T_3$ declines steadily over the seven-hour period. The authors conclude that the binding of $T_3$ to nuclear receptors suppresses TSH in a dose dependent manner, since the occupancy of the $T_3$ nuclear receptors is inversely proportional to the degree of TSH suppression.

These workers also injected 800 ng $T_4$/100 g body weight into hypothyroid rats and reported an increase in nuclear $T_3$, a decrease in
plasma TSH and low serum levels of T_3. These findings indicate that plasma T_4 may also suppress TSH secretion. If $^{125}$I iodinated T_4 and $^{131}$I iodinated T_3 are administered simultaneously to hypothyroid rats, approximately 80 percent of the resultant nuclear bound T_3 is $^{125}$I, thus correlating the cytoplasmic conversion of intrapituitary T_4 and the subsequent nuclear binding of T_3 with the suppression of TSH.

The mechanism by which plasma T_3 and intrapituitary derived T_3 suppress TSH is not known. However, in untreated euthyroid rats 78 percent of the available T_3 nuclear receptor sites are T_3 occupied (50 percent by plasma T_3 and 50 percent by intrapituitary derived T_3). Therefore, both forms of T_3 appear to be required for TSH suppression, and if either plasma T_3 or T_4 fall below a certain critical level pituitary TSH secretion increases.

**The Adenohypophysis**

The hypophysis of the chicken is composed of a distal adenohypophysis, which arises from the dorsal evagination of the pharynx (Rathke's pouch) and a proximal neurohypophysis, derived from the floor of the third ventricle. The adenohypophysis can be further divided into rostral and caudal lobes, and may have seven cell types identifiable histologically by tinctorial methods (74). A tentative classification proposed by Mikami et al. (75, 76) has somatotrophs and prolactin cells as acidophilic and thyrotrophs, gonadotrophs and corticotrophs as basophilic cells. The distribution of specific cell types within the adenohypophysis of the chicken is poorly known. Mikami et al. (77) suggest
that the rostral lobe is composed of prolactin, thyrotropic, corticotrophic and FSH gonadotrophs, while the caudal lobe is made up of somatotroph and LH gonadotroph cells. More recently, Radke and Chiasson (78) reported thyrotrophs to be present in both the rostral and caudal lobes of the chicken pituitary.

Reporting in a series of articles (79-85), Yoshimura and various coworkers suggest that the rat thyrotroph and LH and FSH gonadotrophs are different functional phases of the same basophilic cell. The entire course of the cellular cycle involves eight phases, starting with an immature basophil (Type I). The immature basophil is thought to develop into a Type II cell which is morphologically similar to the presumptive thyrotroph. It is followed by a transitional cell (Type II/III) as the Type II basophil develops into a classical LH gonadotroph (Type III). The Type III basophil may transform into either the typical FSH gonadotroph (Type IV) or a second transitional cell type (Type III/IV). The Type IV and Type III/IV cells, but not the Type II or Type III cells, can either form a retrogressive basophil (Type V) or repeat the cycle by passing through a third intermediate stage (Type IV/II) and reforming a presumptive thyrotroph (Type II). Discovery of the three actively secreting and morphologically distinct intermediate stages has led to the suggestion that thyrotrophs and gonadotrophs are mutually interchangeable. An alternate suggestion explains new immature basophils as arising from chromophobes (81, 86, 87), and still others claim acidophils may be converted to thyrotrophs (80).
The cellular morphology of the adenohypophysis of the chicken (76, 88, 89), white-crowned sparrow (77) and Japanese quail (90-92) has a degree of similarity with the structure of the mammalian adenohypophysis. One important likeness involves the size of the representative thyrotrophs and gonadotrophs. In all species, the LH and FSH gonadotrophs are large spherical or oval shaped cells, while thyrotrophs are small, elongated and sometimes polygonal cells. However, the three intermediate stages described by Yoshimura and coworkers (79-85) have not been demonstrated in the avian pituitary.

The Thyroid Gland and Thyrotropin

The thyroid gland is composed of closed follicles that are lined with epithelial cells surrounding a lumen containing colloid. The number of thyroid follicles and the amount of colloid varies functionally from an inactive gland, where one sees considerable colloid, flat epithelial cells and large follicles to the extremely active gland in which little or no colloid and high cuboidal follicular cells arranged in very small follicles are usual. The gland, therefore, is reduced in size and weight during periods of prolonged inactivity (brought about by high ambient temperature; 93), but increases its DNA content, protein synthesis and actual size and weight during sustained activity (initiated by goitrogen administration; 94).

Synthesis and secretion of thyroid hormones have been extensively investigated in birds and mammals (95, 96). It is well known that thyroglobulin is synthesized by the follicular cells and stored in the
colloid. Binding of TSH to the thyroid cell membrane leads to the reabsorption of thyroglobulin into the follicular cell by an endocytotic process (96). While this glycoprotein is within the follicular cell membrane the tyrosine molecules, bound in peptide linkage to the thyroglobulin, are iodinated by a peroxidase enzyme. Subsequently, once inside the epithelial cell, the peptide bonds holding the iodinated tyrosines to the thyroglobulin are hydrolyzed, by proteolytic enzymes from intracellular lysosomes and released into the cytoplasm. Tri-iodothyronine and/or thyroxine are then released into the systemic circulation while the mono- and di-iodotyrosines are deiodinated by iodotyrosine dehalogenase and the iodine is recycled.

Thyroid stimulating hormone is the primary regulator of $T_3$ and $T_4$ synthesis and secretion. Upon release from the pituitary, TSH binds to specific receptor sites on the plasma membrane of the follicular cells (97, 98, 99, 100), resulting in activation of adenylate cyclase within the cell membrane (101). This enzyme increases intracellular cAMP, which subsequently activates cAMP-dependent protein kinase (102, 103), resulting in the same sequence of events as described for the pituitary. Intracellular activities, such as iodine uptake (104, 105), iodination (106), endocytosis (96), hormone synthesis (106), thyroglobulin breakdown (106) and hormone release (106), may be initiated by TSH binding to membrane receptors or mimicked by exogenous cAMP (105) or its analogue, dibutryl cAMP (104).

Administration of a low iodine diet or goitrogenic compounds causes conspicuous hypertrophy and hyperplasia of the thyroid gland
Methimazole inhibits thyroid hormone synthesis by blocking the iodination of the tyrosine residue and the coupling reaction to form $T_3$ and $T_4$. As a result, the levels of circulating $T_3$ and $T_4$ decrease, the inhibitory influence on the pituitary thyrotroph is removed and plasma TSH levels increase. The elevated TSH concentrations further stimulate the thyroid follicular cells and eventually initiate hypertrophy and hyperplasia of the gland.

**Peripheral Thyroxine 5' Monodeiodination**

The mammalian thyroid gland secretes both $T_3$ and $T_4$, but approximately one-half to four-fifths of the circulating $T_3$ is derived from the 5' monodeiodination of thyroxine in peripheral tissues. The peripheral conversion mechanism has been demonstrated in the liver, kidney and pituitary gland and is thought to be enzymatic; however, the possibility of a chemical nonenzymatic reaction may not be excluded. Preliminary studies have shown that methimazole does not inhibit peripheral deiodination, as does propylthiouracil, $rT_3$ or $3', 5'-T_2$, and that an oxidative mechanism, associated with the microsomal fraction, may be involved in the conversion process.

Since the peripheral conversion of $T_4$ to $T_3$ is a major source of triiodothyronine, inhibition of this important pathway impairs suppression of pituitary TSH secretion and body growth, although plasma $T_4$ may remain within normal limits. Consequently, several workers have concluded that essentially all of the
biological activity of T\textsubscript{4} may be attributed to the T\textsubscript{3} generated by deiodination and that T\textsubscript{4} possesses little biological activity.

**Gonadal Influence on the Hypophysial-Thyroid Axis**

The interactions between the avian gonads and thyroids are more pronounced than in mammals. In mammals, castration initiates only slight effects in thyroid activity, whereas in ducks and quail a strong antagonism has been shown to exist (121, 122). The annual peak in thyroid function generally coincides with seasonal gonadal regression (123, 124) and during the active reproductive period thyroid activity may be depressed (125). Experimental data support these observations, since castration increases thyroid activity and plasma T\textsubscript{4} (121, 126, 127), while goitrogen induced hypothyroidism, or thyroidectomy, increases testicular activity and plasma testosterone in photostimulated birds (126, 128). Tisier-Vidal (121, 122) suggest that castration stimulates pituitary secretion of TSH, resulting in increased levels of thyroid hormone. However, more recent evidence (127) raises serious doubts concerning the validity of this conclusion.

At the peripheral level, the half-life of thyroxine is increased by castration but returns to normal if testosterone is administered (126). These results suggest that testosterone increases the peripheral utilization or urinary excretion of thyroid hormone (129).
Influence of Photoperiod on Hypothalamic-Hypophysial Activity

Photoperiod is known to regulate reproductive activity in birds. It is responsible for determining the onset of the photosensitive phase of the reproductive cycle and for stimulating reproductive development when the bird is in the photosensitive phase (130). Rapid gonadal growth occurs when Japanese quail (131), chickens (132) and tree sparrows (133) are transferred from a short (6 L:18D) to a long (14 L:10D) photoperiod. Evidently, changes in the hypothalamic sensitivity to the negative feedback effects of steroid hormones (134, 135, 136, 137, 138) and the direct stimulating effects of long daily photoperiod (139, 140) are responsible for increased secretion of hypothalamic gonadotropin-releasing hormone. This hypothalamic decapeptide increases pituitary secretion of lutinizing hormone (LH) and follicle stimulating hormone (FSH), resulting in gonadal growth.

In midsummer, even though day lengths are still long, many seasonally breeding species enter the photorefractory phase of the reproductive cycle and terminate reproductive activity. Decreased gonadotropin secretion is believed to initiate gonadal regression (141); however, the mechanism of this response is not known, and is beyond the scope of this report. In contrast to seasonally breeding species, White Leghorn chickens do not become photorefractory in midsummer, and reproductive activity may be maintained throughout the year if the birds are kept on a long daily photoperiod or continuous light (142).
When most photoperiodic species are transferred from photostimulatory (long) to nonphotostimulatory (short) day lengths, the testes become small and aspermic, the androgen-dependent accessory organs regress and plasma gonadotropic levels decrease (135). When these conditions prevail, the bird is considered to be in the photosensitive phase of the reproductive cycle, i.e., if the length of the daily photoperiod increases, a new gonadal cycle will occur. It is suggested that changes in the hypothalamic sensitivity to the negative feedback influence of testosterone are responsible for initiating the photosensitive condition (143).
MATERIALS AND METHODS

Cyclic AMP-Dependent Protein Kinase Assay

Animals were terminated by cervical dislocation and adenohypophysis, thyroid, adrenal glands and a portion of the liver removed and immediately frozen on dry ice. Tissues were then homogenized in either ten volumes (adrenals, liver and thyroids) or 125 μl (adenohypophyses) of a 10 mM sodium-potassium phosphate buffer, pH 6.8, containing 5 mM NaF, 1 mM EDTA, 0.5 mM 3-isobutyl-1-methylxanthine and 150 mM KCl. The homogenate was centrifuged at 4 C for three minutes at 10,000 X g. The supernatant from the liver sample was further diluted to 10 volumes with the homogenization buffer, while 200 μl of the supernatant from the homogenized adrenals was diluted with 50 μl of homogenization buffer. Thyroid and adenohypophysial supernatants were used directly. Five μl of the resulting supernatant from thyroid, adenohypophysis or adrenal preparations and 25 μl of the supernatant of the liver sample were assayed in triplicate for protein kinase activity for three minutes at 30 C both in the presence and absence of saturating amounts of cAMP (10 μM). The 5 μl (25 μl for liver) was added to 10 X 75 mm borosilicate test tubes containing 45 μl (25 μl for liver) of the homogenization buffer. The reaction was initiated by the addition of 25 μl of an assay cocktail resulting in the final assay concentrations: 20 mM sodium-potassium phosphate, pH 6.8, 0.5 mM 3-isobutyl-1-methylxanthine, 50 μg histone F_{11b} (Worthington), 10 mM magnesium chloride, 5 mM NaF.
0.5 µCi of (γ\(^{32}\)P) ATP (5-10 Ci/mMole, New England Nuclear), plus sufficient cold ATP to bring the total concentration to 0.1 mM. Tubes that contained cAMP had a concentration of 10 µM.

The assay was terminated after three minutes at 30 C by pipetting 50 µl of the reaction mixture onto pre-cut strips (1.5 X 5 cm) of instant thin layer chromatography sheets (Gelman) which had been pre-spotted with 70 µl of 20 percent trichloroacetic acid to insure rapid protein denaturation. The spotted strips were chromatographed for 15 minutes in a tank containing a solution of 5 percent trichloroacetic acid plus 0.2 M KCl. This procedure separates the acid insoluble \(^{32}\)PO\(_4\) from unreacted (γ\(^{32}\)P) ATP. The strips were then air-dried and the bottom 3 cm portions separated and placed in 1 ml of a toluene-omnifluor (New England Nuclear) counting fluid. Samples were counted on a liquid scintillation counter (Beckman 230). Forty-five µl of 20 mM EDTA was used in place of the homogenization buffer to determine enzyme blanks. The EDTA chelates the available magnesium ions so the ATP is unable to form the proper substrate for the enzyme. The incorporation of \(^{32}\)PO\(_4\) into histone was linear for protein concentrations up to approximately 60 µg per assay and for five minutes of incubation at 30 C. This procedure is a modification of the assay described by Corbin and Reimann (144).

Cyclic AMP-dependent protein kinase activity in the presence and absence of cAMP was measured at specific time intervals and protein concentrations in all tissues studied. These data were necessary for the proper calculation of homogenization weight to volume ratio in order
to adjust the assay to the linear portion of the scale. Figure 2 illustrates the characteristic activity in the chicken thyroid. Assay time and protein concentration linearity for the liver, adrenal and the adenohypophysis were similar to those of the thyroid.

Cyclic AMP-dependent protein kinase activity was measured as the ratio of $^{32}\text{P}$ incorporated into histone/min/mg protein in the presence and in the absence of exogenous cyclic AMP (−cAMP/+cAMP). The measurement of cAMP-dependent protein kinase in the presence of exogenous cAMP gives an indication of the total intracellular cAMP-dependent protein kinase pool that is present and available for activation. Measurement of cAMP-dependent protein kinase activity in the absence of exogenous cAMP indicates the amount of intracellular cAMP available to activate cAMP-dependent protein kinase in that tissue. An activity ratio of zero indicates no cAMP-dependent protein kinase activity, and a ratio of 1.0 indicates complete activation of the cAMP-dependent protein kinase pool. An intermediate value indicates the degree to which cAMP has been generated within the cell in response to an external hormonal stimulus.

**Hormone Assays**

One hundred μl ($T_4$ assay) or 75 μl ($T_3$ assay) aliquots of chicken plasma were assayed in duplicate by means of the solid phase radioimmunoassay system described by Seth et al. (145). Triiodothyronine derived from the 5' monodeiodination of thyroxine by liver homogenates was determined with a second antibody radioimmunoassay kit (Antibodies
Figure 2. Cyclic AMP-dependent protein kinase activity in the thyroid at various time and enzyme concentrations assayed in the presence (●—●) or absence (○—○) of cyclic AMP.
Incorporated). Since ethanol extracts of tissues contain little or no protein, the only procedural modification made was to add 75 µl of human plasma free of T₃ and T₄ to 25 µl of the ethanol extract sample.

**Formation of Triiodothyronine by Liver Homogenates**

The following technique for determining the 5' monodeiodination of thyroxine by liver tissue homogenates was described by Chopra (11). Liver tissue was removed, washed in cold (6 C) 0.15 M phosphate buffer, pH 7.35, blotted, weighed and homogenized in two volumes (w/v) of 0.15 M phosphate buffer by means of a glass homogenizer. The homogenate was filtered through four layers of cheesecloth and stored at -20 C before assay. The homogenates were composed of approximately 0.33 grams wet tissue weight/ml buffer, which may also be expressed as 0.33 gram-equivalent (g-eq) of tissue.

Four hundred µl of tissue homogenate were added to each of two 13 X 100 mm test tubes containing 100 µl of a solution containing 50 µg T₄/ml and 500 µl of 0.15 M phosphate buffer, pH 7.35. The tubes were thoroughly mixed by swirling and placed in a shaker water bath (Dubnoff) for 120 minutes at 37 C. The reaction was stopped by the addition of 2 ml of 95% ethanol. Each assay included two zero incubation tubes which were handled as described above, except that 400 µl of homogenate were added at the end of the two-hour incubation period immediately prior to extraction with ethanol. The T₃ present in the ethanol extract of these tubes represents the T₃ found in the tissue homogenate plus the T₃ contamination of the commercially prepared T₄ (Sigma). The
amount of $T_3$ present in these tubes was subtracted from the $T_3$ value of the test samples to determine the amount of $T_3$ generated by incubation of the liver homogenate. The amount of $T_3$ found in the ethanol extract was measured by radioimmunoassay and expressed as ng $T_3$ generated/h/g-eq tissue.

**Protein and DNA Determination**

Wet weights of all tissues were determined on a torsion balance (Roller-Smith). Protein and DNA were determined by absorbance changes measured on a spectrophotometer (Beckman DU-2). Protein was assessed by the Coomassie Blue G 250 method described by Bradford (146), in which bovine serum albumin is used as a standard. Pituitary and thyroid deoxyribonucleic acid (DNA) values were determined by the modified diphenylamine method described by Giles and Myers (147), in which calf thymus DNA (Sigma) serves as a standard.

**Histology**

Adenohypophyses and thyroids were mordanted in either 10% buffered formalin or Hollande-Bouins fluid, sectioned at 5 microns and stained with hematoxylin and eosin (148). Slides were examined microscopically and photographed with a Zeiss Photomicroscope.

**Statistics**

All data except that pertaining to the effects of ambient temperature were analyzed by a one-way analysis of variance with an LSD test for significance. A two-way analysis of variance with an LSD test for significance was used to test the effects of different ambient
temperatures on hypothyroid, euthyroid and castrated cockerels. Only values at the 0.05 level or better were considered to be significant.

**Animal Procedures**

**Animal Maintenance**

White Leghorn chickens hatched at the University of Arizona Poultry Research Center were used in all of the experiments described in this study. The animals were maintained in brooder cages and fed a University of Arizona chick starter mash until six weeks of age. The birds were then transferred to sheltered ground pens or suspended laying cages and fed a University of Arizona laying mash and water *ad libitum*. All birds were housed in suspended laying cages for a minimum of ten days prior to the initiation of an experiment.

**Castration**

Five week old male White Leghorns were deprived of food for 48 h and water for 24 h prior to castration. At the time of surgery, the birds were placed on their side on an operating board and restrained by tying down the legs and wings. An incision was made between the fifth and sixth vertebral rib, a rib spreader was inserted and the air sacs punctured with a surgical hook. A single testis was removed and the incision closed with 9 mm Autoclips (Clay Adams). The bird was turned over and an incision made on the opposite side through which the other testis was removed. Postoperatively, the animals were routinely checked for "wind puffs" and deflated by incising the skin. Animals were allowed food and water *ad libitum*. 
Hormone and Drug Administration

Five centimeter strips of silastic tubing (Dow Corning, 0.062 in. I.D. X 0.095 in. O.D.) filled with testosterone propionate (Sigma) and sealed with a medical grade adhesive (Dow Corning) were inserted subcutaneously and the incision closed with 9 mm Autoclips (Clay Adams). Implants were changed every 14 days.

Methimazole (Sigma) was administered either orally, 0.1% mixed with the food, or by a 0.5 ml daily subcutaneous injection of a 0.9% saline solution containing 100 mg methimazole per milliliter.

Thyrotropin releasing hormone (TRH, Sigma) was injected in a wing vein. The hormone was prepared in a 0.9% saline solution containing 200 μg TRH/ml and administered in 0.5 ml aliquots.

Experimental Procedures

Cyclic AMP-Dependent Protein Kinase Activity in Prepubertal and Old Adult Chicks

Four groups of ten birds each were arranged as follows: Group I, young males aged 13-17 weeks; Group II, young females aged 13-17 weeks; Group III, retired male breeders aged 20-22 months; and Group IV, retired female breeders aged 22-25 months. Five animals in each group were injected with 0.5 ml of 0.9% saline into the wing vein and the other five were injected with the TRH solution. Thirty minutes after injection, the adenohypophysis, thyroid, liver and adrenal glands of all birds (except the retired male breeders) were removed and assayed for cAMP-dependent protein kinase activity. Only the adenohypophysis and thyroid glands of old males were investigated.
Temporal Changes in Adenohypophysial and Thyroid Activity After TRH Administration

Fifty 12-15 week old hens were divided into ten groups of five birds each. Group I birds represented zero time controls and were injected with 0.5 ml of 0.9% saline in a wing vein. Groups II-X were injected intravenously with the TRH solution and sacrificed at ten or twenty minute intervals after injection. At the time of sacrifice 3-5 ml blood samples were taken from a wing vein in heparinized syringes, except at ten minutes after TRH injection. These samples were centrifuged and the plasma stored at -20 C prior to $T_3$ and $T_4$ radioimmuno-assay. The adenohypophysis and thyroid glands were removed and assayed for cAMP-dependent protein kinase activity.

Liver Conversion of Thyroxine to Tri-iodothyronine at Selected Intervals After TRH Administration

Thirty 12-15 week old hens were divided into six groups of five birds each. Group I birds were injected with 0.5 ml of 0.9% saline in a wing vein and represented zero time controls. Group II-VI hens were injected in a wing vein with the TRH solution and sacrificed at 30, 60, 90, 120 or 180 minutes postinjection. A liver sample was assayed for its ability to deiodinate thyroxine to triiodothyronine.

Pituitary and Thyroid Morphology of Untreated, Castrated and Hypothyroid Cockerels

Twenty cockerels from a flock of 40 male birds were castrated at five weeks of age and allowed to recover for two weeks. At seven
weeks of age ten castrated and ten of the noncastrated birds were fed 0.1% methimazole in a standard University of Arizona laying mash. The remaining ten castrated and ten unoperated cockerels were fed the standard University of Arizona laying mash. All animals were allowed water ad libitum and subjected to environmental temperature fluctuations from 21 C to 38 C with an average daily temperature of 27 C. At 17 weeks of age the cockerels were weighed and sacrificed by cervical dislocation. The adenohypophyses were removed, weighed and either frozen on dry ice (for quantitation of DNA and protein) or mordanted in 10% formalin for histological sectioning. The thyroids were frozen on dry ice and assayed for DNA and protein content.

Hypothyroid, Euthyroid and/or Castrated Cockerels Subjected to Ambient Temperature Extremes

Forty-eight cockerels were divided into four groups of twelve birds each. Twenty-four of these cockerels were castrated at five weeks of age and allowed two weeks to recover from surgery. At seven weeks of age 12 castrated and 12 normal cockerels were fed a standard University of Arizona laying mash containing 0.1% methimazole. The remaining 12 castrates and 12 intact cockerels were fed only the standard University of Arizona laying mash. The four groups of birds were placed in individual cages in an environmental chamber (Scherer) at 24 C ± 1 C with a lighting schedule of 14 h light and 10 h darkness (14L:10D). They were maintained for ten weeks and sacrificed by cervical dislocation. At the time of termination, total body, adenohypophysis, thyroid and adrenal
weights were determined, thyroids were mordanted for histological sectioning and cAMP-dependent protein kinase activities in the adeno-hypophysis, thyroid and liver tissues were assayed.

A second hatch of 48 cockerels was divided into four groups as above and placed in the environmental chamber (Scherer) at seven weeks of age. The chamber was maintained at 6 C ± 1 C with a lighting schedule of 14L:10D. This hatch was also held for ten weeks and sacrificed by cervical dislocation. Body, adenohypophysis, thyroid and adrenal weights were recorded, thyroids fixed and cAMP-dependent protein kinase activities in the adenohypophysis, thyroid and liver tissues assayed.

At seven weeks of age a third hatch of 48 cockerels was divided into four groups and maintained for ten weeks in individual cages in the environmental chamber (Scherer) at 33 C ± 1 C with 14L:10D. One castrated and one intact group of cockerels were given daily subcutaneous injections of methimazole. Both groups were fed a standard University of Arizona laying mash along with the untreated and castrated groups. At 17 weeks of age the birds were sacrificed by cervical dislocation and gland and body weights were determined. Adenohypophysis, thyroid and liver tissues from these birds were assayed for cAMP-dependent protein kinase activities and a portion of each thyroid was prepared for histological study.

Temporal Changes in Pituitary and Thyroid Activity of Castrated Cockerels Subjected to Different Photoperiods

At the time of castration, twelve cockerels received subcutaneous implants of testosterone propionate and were placed in individual cages
in an environmental chamber. These birds were then maintained for 11 weeks at 24°C ± 1°C with a lighting schedule of 14L:10D. At 16 weeks of age the implants were removed, and three cockerels were sacrificed and assayed for pituitary and thyroid cAMP-dependent protein kinase activity. Every seven days thereafter three birds were terminated and the pituitary and thyroid glands assayed for cAMP-dependent protein kinase activity, until 21 days had elapsed.

Twenty cockerels from an additional hatch were castrated and given testosterone propionate implants. These 20 birds and five untreated controls from the same hatch were maintained in the environmental chamber at 24°C ± 1°C with a lighting schedule of 6L:10D. These birds were provided food and water ad libitum for ten weeks. Testosterone implants were removed at 15 weeks of age and five cockerels were terminated and their adenohypophysis and thyroid glands assayed for cAMP-dependent protein kinase activity. Every seven days thereafter, five birds were terminated and their glands assayed, until 21 days had elapsed. The five untreated controls were terminated and their glands assayed at the end of the experimental period.
RESULTS

Activity of the Hypophysial-Thyroid Axis in Response to Thyrotropin Releasing Hormone

Activation in Prepubertal and Old Adult Chickens

Immature and aged chickens of both sexes had similar pituitary cAMP-dependent protein kinase activity ratios. However, the cAMP-dependent protein kinase pool was much greater in the old males than in the other three groups (Table 1).

The adenohypophysis of young males and young females responded maximally 30 minutes after an injection of TRH, resulting in a significant cAMP-dependent protein kinase activity ratio increase over the unstimulated birds. The adenohypophysis of old males and females did not vary significantly in activity following TRH injection (Figure 3).

The thyroid protein kinase pools and activity ratios were similar in unstimulated young birds of both sexes. These values were also similar in both sexes of old birds, but were significantly greater than in the immature birds (Figure 4). The thyroids of young birds and old females responded to TRH with a significant increase in their protein kinase activity ratio (Table 1).

The cAMP-dependent protein kinase activity ratios were significantly higher in the livers of all birds that received TRH injections (Figure 5 and Table 2). The adrenal protein kinase activity ratio in
Table 1.

Cyclic AMP-dependent protein kinase alterations in the thyroid and pituitary glands following administration of 0.9% saline or 100 μg TRH.

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<th></th>
<th>PITUITARY</th>
<th>THYROID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>TRH Injected</td>
</tr>
<tr>
<td></td>
<td>-cAMP</td>
<td>+cAMP</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1588</td>
<td>3245</td>
</tr>
<tr>
<td>n=5</td>
<td>±114</td>
<td>±169</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Young</td>
<td>1573</td>
<td>3311</td>
</tr>
<tr>
<td>Females</td>
<td>±105</td>
<td>±164</td>
</tr>
<tr>
<td>n=5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>2265</td>
<td>4624**</td>
</tr>
<tr>
<td>Males</td>
<td>±131</td>
<td>±139</td>
</tr>
<tr>
<td>n=4</td>
<td></td>
<td></td>
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<tr>
<td>Old</td>
<td>1705</td>
<td>3641</td>
</tr>
<tr>
<td>Females</td>
<td>±124</td>
<td>±109</td>
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<td>n=5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) = are significantly different from their untreated values (p < 0.05).
(**) = are significantly different from the other treatment groups (p < 0.05).
Figure 3. Pituitary cAMP-dependent protein kinase activity ratio 30 minutes after administration of 0.9% saline or 100 μg TRH. Vertical lines represent ± SEM.
Figure 4. Thyroid cAMP-dependent protein kinase activity ratio 30 minutes after administration of 0.9% saline or 100 μg TRH. Vertical lines represent ± SEM.
Figure 5. Liver cAMP-dependent protein kinase activity ratio 30 minutes after administration of 0.9% saline or 100 µg TRH. Vertical lines represent ± SEM.
Cyclic AMP-dependent protein kinase alterations in the liver and adrenal glands following administration of 0.9% saline or 100 μg TRH.

<table>
<thead>
<tr>
<th></th>
<th>LIVER</th>
<th>ADRENAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control TRH Injected</td>
<td>Control TRH Injected</td>
</tr>
<tr>
<td></td>
<td>-cAMP +cAMP -/+</td>
<td>-cAMP +cAMP -/+</td>
</tr>
<tr>
<td>Young Males n=5</td>
<td>1454 ±145 2496 ±285 0.59 ±1.01 1549 ±59 1953 ±101 0.80±0.04</td>
<td>1509 ±157 3147 ±159 0.48 ±1.04 1666 ±142 3139 ±166 0.46 ±0.03</td>
</tr>
<tr>
<td>Young Females n=5</td>
<td>1750 ±175 0.54 ±1.02 1459* ±137 1669 ±96 0.87* ±1.03</td>
<td>1945 ±103 3902 ±138 0.50 ±1.02 1768 ±114 3740 ±137 0.47 ±0.02</td>
</tr>
<tr>
<td>Old Males n=4</td>
<td>---- ---- ---- ---- ---- ----</td>
<td>---- ---- ---- ---- ---- ----</td>
</tr>
<tr>
<td>Old Females n=5</td>
<td>943 ±94 1750 ±88 0.54 ±1.02 1459* ±137 1669 ±96 0.87* ±1.03</td>
<td>1945 ±103 3902 ±138 0.50 ±1.02 1768 ±114 3740 ±137 0.47 ±0.02</td>
</tr>
</tbody>
</table>

(*) = significantly different from their untreated values (p < 0.05).
the young females was greater than in the young males or old females and did not change with TRH administration (Table 2).

Temporal Changes in Cyclic AMP-Dependent Protein Kinase Activity

Ten minutes after injection of TRH the adenohypophysial cAMP-dependent protein kinase activity ratio was increased slightly but significantly (Figure 6). Peak activity was achieved after 20 minutes and a plateau was maintained up to 80 minutes postinjection. Subsequently, a sharp decline in activity occurred and basal levels were reinstated approximately two hours after injection of the neurohormone (Figure 6).

Thyroid cAMP-dependent protein kinase activity achieved maximal elevation 10 minutes after injection of TRH and remained maximally elevated until 80 minutes postinjection. Activity then gradually declined, when by four hours the protein kinase activity ratio returned to baseline values (Figure 6).

Temporal Changes in Plasma Thyroxine and Triiodothyronine

Sixty minutes after injection of TRH the plasma thyroxine concentration reached peak levels following which it then declined rapidly, reaching preinjection levels 100 minutes after initial TRH administration (Figure 7). Plasma triiodothyronine increased to peak levels 80 minutes after TRH injection, a sharp decline followed for the next 40 minutes, resulting in reestablishment of preinjection levels by 140 minutes (Figure 7).
Figure 6. Temporal changes in the adenohypophysial (●—●) and thyroid (○—○) cAMP-dependent protein kinase activity ratio following TRH administration. Vertical lines represent ± SEM.
Figure 7. Temporal changes in the plasma concentrations of thyroid hormones and liver thyroxine 5' monodeiodinating activity following administration of 100 µg TRH. The vertical lines represent ± SEM.
Conversion of Thyroxine to Tri-iodothyronine by Liver Homogenates

Sixty minutes after injection of TRH the ability of the liver homogenates to monodeiodinate thyroxine to triiodothyronine was increased to peak levels. The level was maintained until two hours post-injection and then declined, returning to control levels by three hours after initial injection (Figure 7).

Body and Gland Weights of Euthyroid, Hypothyroid and Castrated Cockerels at Three Ambient Temperatures

Body Weights

The three ambient temperatures used in this study (6 C, 24 C and 33 C) resulted in marked differences in body size and appearance of both castrated cockerels and those fed methimazole (Figure 8). The gross appearance of the methimazole-fed cockerels was not altered by castration. Castrated birds had characteristically reduced combs and wattles and pale rather than bright yellow beaks as in normal birds. Both castrated and intact cockerels fed methimazole had reduced combs and wattles and pale colored bills similar to the castrates. However, the combs and wattles of the two groups fed methimazole were miniatures of the characteristic single comb male with spikes, whereas the castrates had combs similar to the single comb female without spikes.

Control cockerels were always heavier than methimazole-fed birds (whether castrated or not), and the body weights of castrates were similar to those of controls at all ambient temperatures studied. Both untreated and castrated cockerels exhibited the greatest weight gain when maintained at 24 C. Methimazole-fed birds (whether castrated or
Figure 8. Seventeen-week-old cockerels maintained at 24 C for 10 weeks following castration and/or methimazole treatment. (A) Untreated cockerel, (B) Castrated cockerel, (C) Castrated cockerel fed methimazole, and (D) Methimazole-fed cockerel.
Figure 8—Continued
not) had similar body weights at the three ambient temperatures, but were always significantly smaller than castrates or untreated birds (Figure 9 and Table 3).

Adenohypophysial Weights

Regardless of ambient temperature, the castrated cockerels had adenohypophysial glands that were twice as heavy as those of untreated birds. Methimazole-fed cockerels had pituitaries significantly smaller than control birds (Figure 10). At 6 C, 24 C and 33 C, the castrated cockerels fed methimazole had adenohypophysial glands that weighed the same as controls, but were significantly different from both the castrates and the methimazole-fed group (Figure 10 and Table 3).

Thyroid Weights

The thyroid glands of untreated cockerels subjected to 6 C weighed 125 ± 7 mg; cockerels held at 24 C had thyroids weighing 93 ± 3 mg; and those held at 33 C had thyroids weighing only 73 ± 5 mg. Castrated cockerels held in the cold also exhibited thyroid glands much heavier than castrated birds subjected to higher ambient temperatures (24 C and 33 C). However, unlike untreated cockerels, the thyroid glands of castrated birds weighed the same at 24 C and 33 C (Figure 11 and Table 3). Methimazole-fed cockerels (whether castrated or not) had significantly larger thyroid glands than did controls at all ambient temperatures.
Figure 9. Body weights of 17-week-old cockerels maintained at either 6 C, 24 C, or 33 C for 10 weeks following castration and/or methimazole administration. Vertical lines represent ± SEM.
Table 3.

Body, pituitary, thyroid and adrenal weights of 17-week-old cockerels maintained at either 6 C, 24 C, or 33 C for 10 weeks following castration and/or methimazole administration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Pituitary Weight (mg)</th>
<th>Thyroid Weight (mg)</th>
<th>Adrenal Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 C</td>
<td>1368&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>24 C</td>
<td>1769&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>33 C</td>
<td>1321&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
</tbody>
</table>

| Castrate           |                 |                       |                     |                     |
| 6 C                | 1336<sup>a</sup> | 17.9<sup>b</sup>      | 138<sup>a</sup>     | 171<sup>b</sup>     |
| 24 C               | 1634<sup>a</sup> | 18.9<sup>b</sup>      | 86<sup>a</sup>      | 106<sup>b</sup>     |
| 33 C               | 1308<sup>a</sup> | 16.2<sup>b</sup>      | 88<sup>a</sup>      | 108<sup>b</sup>     |
| (n)                | (5)             | (10)                  | (10)                | (10)                |

| Castrate + Methimazole |                 |                       |                     |                     |
| 6 C                | 798<sup>b</sup> | 10.9<sup>a</sup>      | 494<sup>b</sup>     | 87<sup>c</sup>      |
| 24 C               | 869<sup>a</sup> | 10.1<sup>a</sup>      | 243<sup>b</sup>     | 63<sup>b</sup>      |
| 33 C               | 929<sup>a</sup> | 10.0<sup>a</sup>      | 358<sup>b</sup>     | 86<sup>a</sup>      |
| (n)                | (7)             | (10)                  | (10)                | (10)                |

| Methimazole        |                 |                       |                     |                     |
| 6 C                | 742<sup>b</sup> | 7.3<sup>c</sup>       | 419<sup>b</sup>     | 100<sup>c</sup>     |
| 24 C               | 891<sup>a</sup> | 6.8<sup>c</sup>       | 256<sup>b</sup>     | 86<sup>a</sup>      |
| 33 C               | 895<sup>a</sup> | 7.0<sup>c</sup>       | 316<sup>b</sup>     | 85<sup>b</sup>      |
| (n)                | (6)             | (10)                  | (10)                | (10)                |

Means in vertical columns that have the same superscript are not significantly different (p > 0.05). Means in horizontal rows with the same subscript are not significantly different (p > 0.05).
Figure 10. Pituitary weights of 17-week-old cockerels maintained at either 6 C, 24 C, or 33 C for 10 weeks following castration and/or methimazole administration. Vertical lines represent ± SEM.
Figure 11. Thyroid weights of 17-week-old cockerels maintained at either 6 C, 24 C or 33 C for 10 weeks following castration and/or methimazole administration. Vertical lines represent ± SEM.
Adrenal Weights

Untreated cockerels held at high ambient temperatures (33 C) had the smallest adrenal glands, while the cockerels held at low (6 C) or moderate (24 C) ambient temperatures had adrenals that were equal in size (Figure 12 and Table 3). Castrated cockerels maintained at a low ambient temperature had the heaviest adrenal glands, but when subjected to moderate (24 C) or high (33 C) temperatures the adrenals were the same weight.

Methimazole-fed cockerels (whether castrated or not) had significantly smaller adrenal glands than control birds. These two groups of birds did not exhibit significant change in adrenal weight with changes in ambient temperature.

Cyclic AMP-Dependent Protein Kinase Activity in Euthyroid, Hypothyroid and Castrated Cockerels at Three Ambient Temperatures

Adenohypophysis

The adenohypophysial cAMP-dependent protein kinase activity ratios in cockerels held at a moderate (24 C) temperature were similar in all treatment groups (Figure 13). However, the protein kinase pool was significantly lower in the pituitaries of castrated cockerels (whether fed methimazole or not) than in the other groups (Table 4).

Adenohypophysial cAMP-dependent protein kinase activity was higher in all birds during cold stress (6 C) than at 24 C; however, this difference was not significant in methimazole-fed castrates (Figure 13 and Table 4). At 6 C, the highest protein kinase activity ratio was
Figure 12. Adrenal weights of 17-week-old cockerels maintained at either 6 C, 24 C or 33 C for 10 weeks following castration and/or methimazole administration. Vertical lines represent ± SEM.
Figure 13. Pituitary cAMP-dependent protein kinase activity ratio in 17-week-old cockerels maintained at either 6°C, 24°C, or 33°C for 10 weeks following castration and/or methimazole administration. Vertical lines represent ± SEM.
Table 4.
Temperature alterations of pituitary cAMP-dependent protein kinase in euthyroid, hypothyroid and castrated cockerels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6 C</th>
<th>24 C</th>
<th>33 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-cAMP</td>
<td>+cAMP</td>
<td>-/+</td>
</tr>
<tr>
<td>Control</td>
<td>1765&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;b&lt;/sub&gt; ±153 (5)</td>
<td>2776&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±173 (8)</td>
<td>0.64&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±296 ±0.03 (5)</td>
</tr>
<tr>
<td>Castrate</td>
<td>1322&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±94 (4)</td>
<td>2463&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;b&lt;/sub&gt; ±103 (5)</td>
<td>0.54&lt;sup&gt;c&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±213 ±0.02 (5)</td>
</tr>
<tr>
<td>Castrate + Methimazole</td>
<td>1445&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;b&lt;/sub&gt; ±206 (4)</td>
<td>2873&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±64 (5)</td>
<td>0.50&lt;sup&gt;c&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±289 ±0.03 (5)</td>
</tr>
<tr>
<td>Methimazole</td>
<td>1849&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±159 (5)</td>
<td>2248&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±188 (7)</td>
<td>0.82&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt; ±175 ±0.03 (5)</td>
</tr>
</tbody>
</table>

( ) Number of repetitions.

Treatment means, column values for a specific temperature, that have the same superscript are not significantly different (p > 0.05).

Temperature means, row values for a specific treatment, that have the same subscript are not significantly different (p > 0.05).
seen in the intact methimazole-fed castrates. The controls and castrates had activity ratios that were intermediate to the castrated and noncastrated methimazole-fed groups. Since the +cAMP values were similar in all four groups maintained at 6 C, castration and methimazole treatment does not appear to affect the total protein kinase pool. Therefore, alterations in the activity ratio were due to a greater activation of the available protein kinase (Table 4).

Castrates (whether fed methimazole or not) had a greater adenohypophysial cAMP-dependent protein kinase activity ratio at 33 C than at 24 C. Cockerels in all four groups held at high ambient temperatures (33 C) had smaller protein kinase pools than did those birds held at 24 C (Table 4). However, the pituitary pools were not significantly different in the various groups held at 33 C. Therefore, castration or methimazole treatment does not appear to affect the total pituitary protein kinase pool of birds held at 33 C.

The adenohypophyses of castrated cockerels maintained at 33 C had higher activity ratios than those of the control group. The methimazole-fed birds had activity ratios similar to those of untreated cockerels and the castrated birds fed methimazole had activity ratios similar to both controls and castrates (Table 4). Thus, methimazole administration reduced the castration induced increase in the adenohypophysial cAMP-dependent protein kinase activity ratio.

Thyroid

Untreated cockerels had thyroid cAMP-dependent protein kinase activity ratios that were below 0.60 at 24 C and 33 C. However, at
24 C and 33 C the castrated and/or methimazole treated cockerels had activity ratios that were significantly higher (from 0.70 to 0.86) than the untreated birds (Figure 14 and Table 5).

Liver

The four treatment groups had similar liver cAMP-dependent protein kinase pools and activity ratios at each of the ambient temperatures investigated. When the activity ratios of all birds, regardless of treatment received, at either 6 C, 24 C or 33 C were averaged and compared, the cockerels held at 33 C had a higher mean activity ratio than the birds maintained at the other ambient temperatures (Table 6).

Pituitary and Thyroid Morphology of Euthyroid, Hypothyroid and Castrated Cockerels

Pituitary

Figure 15 presents photomicrographs of midsaggital sections of the adenohypophysis from each of the four treatment groups. The pituitaries from untreated cockerels or castrates fed methimazole were intermediate in size to pituitaries from castrates or methimazole-fed cockerels.

The nuclei of the pituitary cells were similar in size but the amount of cytoplasm varies considerably between cell types. The pituitary cells of castrated birds were more chromophobic and have uniformly more cytoplasm than the pituitary cells of the other cockerels. The pituitaries of methimazole-fed cockerels were composed of smaller cells than the pituitaries of the other groups (Figure 16).
Figure 14. Thyroid cAMP-dependent protein kinase activity ratio in 17-week-old cockerels maintained at either 6°C, 24°C or 33°C for 10 weeks following castration and/or methimazole administration. Vertical lines represent ± SEM.
Table 5.

Temperature alterations of thyroid cAMP-dependent protein kinase in euthyroid, hypothyroid and castrated cockerels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6 C</th>
<th>24 C</th>
<th>33 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-cAMP</td>
<td>+cAMP</td>
<td>-/+</td>
</tr>
<tr>
<td>Control</td>
<td>550&lt;sup&gt;a&lt;/sup&gt;</td>
<td>801&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>±58</td>
<td>±103</td>
<td>±.04</td>
</tr>
<tr>
<td>Castrate</td>
<td>628&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>786&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>±63</td>
<td>±64</td>
<td>±.05</td>
</tr>
<tr>
<td>Castrate + Methimazole</td>
<td>626&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>931&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>±47</td>
<td>±42</td>
<td>±.05</td>
</tr>
<tr>
<td>Methimazole</td>
<td>800&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>±108</td>
<td>±78</td>
<td>±.07</td>
</tr>
</tbody>
</table>

( ) Number of repetitions.

Treatment means, column values for a specific temperature, that have the same superscript are not significantly different (p > 0.05).

Temperature means, row values for a specific treatment, that have the same subscript are not significantly different (p > 0.05).
Table 6.

Temperature alterations of liver cAMP-dependent protein kinase in euthyroid, hypothyroid and castrated cockerels.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Castrate</th>
<th>Castrate + Methimazole</th>
<th>Methimazole</th>
<th>Means of All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1296 ±88</td>
<td>782 ±151</td>
<td>1181 ±105</td>
<td>1211 ±192</td>
<td>0.59 ±192</td>
</tr>
<tr>
<td></td>
<td>2064 ±127</td>
<td>1944 ±149</td>
<td>1945 ±170</td>
<td>1802 ±166</td>
<td>0.55 ±166</td>
</tr>
<tr>
<td></td>
<td>1364 ±47</td>
<td>1036 ±45</td>
<td>1304 ±135</td>
<td>1021 ±97</td>
<td>0.55 ±97</td>
</tr>
<tr>
<td></td>
<td>2450 ±109</td>
<td>1919 ±129</td>
<td>1970 ±103</td>
<td>1793 ±146</td>
<td>0.55 ±146</td>
</tr>
<tr>
<td></td>
<td>0.64 ±.05</td>
<td>0.51 ±.01</td>
<td>0.59 ±.03</td>
<td>0.65 ±.05</td>
<td>0.59 ±.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.56 ±.01</td>
<td>0.57 ±.02</td>
<td>0.55 ±.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.60 ±.04</td>
<td>0.72 ±.06</td>
<td>0.72 ±.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

( ) Number of repetitions.

Treatment means with the same superscript are not significantly different (p > 0.05).
Figure 15. Midsaggital sections of the adenohypophysis of 17-week-old cockerels (10 x). (A) Untreated cockerel, (B) Castrated cockerel, (C) Castrated cockerel fed methimazole, and (D) Methimazole-fed cockerel. Note the difference in size and intensity of staining.
Figure 15--Continued
Figure 16. Adenohypophysis of 17-week-old untreated cockerel (X 200). Note variability in dispersal of nuclei.

Figure 17. Adenohypophysis of 17-week-old castrated cockerel (X 200). Note widely dispersed nuclei, abundant cytoplasm and poor staining.
Figure 18. Adenohypophysis of 17-week-old cockerel fed methimazole (X 200). Note large aggregations of nuclei, small amounts of cytoplasm and intense staining.

Figure 19. Adenohypophysis of 17-week-old castrated cockerel fed methimazole (X 200). Note wide dispersal of nuclei in some areas and close aggregations in other areas.
Table 7 presents the protein and DNA content of the pituitary glands of the four groups of cockerels. Untreated and castrated cockerels had similar concentrations of DNA and protein, but methimazole-fed birds (whether castrated or not) had approximately one-half the DNA content of the other groups. The protein content of pituitaries from methimazole-fed castrates was one-third less than that of the other three groups.

Due to the increased size of their pituitary gland, the DNA/mg gland and protein/mg gland values were much lower in castrated than in control cockerels. In contrast, because of their reduced pituitary size, these ratios were much higher in methimazole-fed than in castrated birds; however, only protein/mg gland values were significantly higher than control (Table 7).

Thyroid

The thyroid gland of methimazole-fed cockerels had considerably more DNA than the thyroids of the other three groups. The two methimazole-fed groups had enlarged thyroid glands, reduced total protein and consequently less protein/mg gland than either untreated or castrated cockerels (Table 8).

The thyroid gland exhibited marked histological changes with methimazole administration but only subtle differences occurred with castration. The thyroid glands of untreated cockerels maintained at 24 C are composed of large colloid filled follicles with squamous follicular cells and few reabsorption lacunae (Figure 20). The thyroids
Table 7.
DNA and protein in the pituitary of castrated and/or goitrogen treated cockerels.

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>μg DNA/Gland</th>
<th>μg Dna/mg Gland</th>
<th>μg Protein/Gland</th>
<th>μg Protein/mg Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(6)</td>
<td>63.6 ± 2.7\textsuperscript{a}</td>
<td>5.83 ± 0.50\textsuperscript{a}</td>
<td>340 ± 19.3\textsuperscript{a}</td>
<td>32.1 ± 2.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Castrate</td>
<td>(5)</td>
<td>65.6 ± 4.6\textsuperscript{a}</td>
<td>3.53 ± 0.19\textsuperscript{b}</td>
<td>388 ± 24.2\textsuperscript{a}</td>
<td>21.6 ± 1.8\textsuperscript{b}</td>
</tr>
<tr>
<td>Castrate + Methimazole</td>
<td>(4)</td>
<td>36.0 ± 3.2\textsuperscript{b}</td>
<td>4.61 ± 0.30\textsuperscript{ab}</td>
<td>220 ± 23.1\textsuperscript{b}</td>
<td>28.6 ± 3.8\textsuperscript{ab}</td>
</tr>
<tr>
<td>Methimazole</td>
<td>(5)</td>
<td>37.6 ± 1.9\textsuperscript{b}</td>
<td>6.00 ± 0.69\textsuperscript{a}</td>
<td>363 ± 24.2\textsuperscript{a}</td>
<td>53.8 ± 5.3\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c} Means in a vertical column with the same superscript letters are in the same subset (ANOVA).
Table 8.

DNA and protein in the thyroid gland of castrated and/or goitrogen treated cockerels.

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>µg DNA/Gland</th>
<th>µg DNA/mg Gland</th>
<th>µg Protein/Gland</th>
<th>µg Protein/mg Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(5)</td>
<td>232.8 ± 39.8a</td>
<td>1.98 ± 0.22a</td>
<td>7017 ± 714a</td>
<td>60.2 ± 5.8a</td>
</tr>
<tr>
<td>Castrate</td>
<td>(5)</td>
<td>357.4 ± 21.3a</td>
<td>3.23 ± 0.56ab</td>
<td>5450 ± 833a</td>
<td>46.6 ± 5.9b</td>
</tr>
<tr>
<td>Castrate + Methimazole</td>
<td>(4)</td>
<td>409.5 ± 80.5a</td>
<td>3.33 ± 0.43ab</td>
<td>2930 ± 316b</td>
<td>24.8 ± 2.8c</td>
</tr>
<tr>
<td>Methimazole</td>
<td>(5)</td>
<td>673.2 ± 121.5b</td>
<td>3.87 ± 0.74b</td>
<td>4817 ± 1016ab</td>
<td>25.4 ± 0.3c</td>
</tr>
</tbody>
</table>

Means in a vertical column with the same superscript letter are in the same subset (ANOVA).
Figure 20. Thyroid gland of 17-week-old untreated cockerel at 24 C (X 100). The presence of large amounts of colloid and squamous follicle cells is evident.

Figure 21. Thyroid gland of 17-week-old castrated cockerel at 24 C (X 100). Colloid is abundant and follicle cells are squamous.
Figure 22. Thyroid gland of 17-week-old methimazole-fed cockerel at 24 C (X 100). Hypertrophy and hyperplasia of the follicle cells and a reduction in the amount of colloid.

Figure 23. Thyroid gland of 17-week-old castrated cockerel fed methimazole at 24 C (X 100). Hypertrophy and hyperplasia of follicle cells and increases in colloid are greater at the edge than in the middle of the gland.
Figure 24. Thyroid gland of 17-week-old untreated cockerel at 6 C (X 100). Large amounts of colloid, few reabsorption lacunae and hypertrophy of follicle cells.

Figure 25. Thyroid gland of 17-week-old castrated cockerel at 6 C (X 100). Enlarged follicle cells and abundant colloid.
Figure 26. Thyroid gland of 17-week-old methimazole-fed cockerel at 6 C (X 100). Note absence of colloid and large numbers of re-absorption lacunae in the remaining colloid. Follicle cells are hypertrophied.

Figure 27. Thyroid gland of 17-week-old castrated cockerel-fed methimazole at 6 C (X 100). Abundant colloid and hypertrophied follicle cells.
of castrated cockerels are similar to those of controls (Figure 21). Methimazole-fed cockerels have thyroid glands with high cuboidal follicular cells and reduced colloid. The reduction in follicular colloid leads to a folding of the follicular walls and in extreme instances the gland appears as a solid mass of cells (Figure 22). The thyroids of castrated cockerels fed methimazole have high cuboidal cells, greater amounts of follicular colloid, especially in the peripheral follicles, and more reabsorption lacunae (Figure 23) than the thyroids of intact methimazole-fed cockerels.

The thyroid follicular cells of untreated and castrated cockerels held at low (6°C) ambient temperature are high cuboidal and the colloid has many reabsorption lacunae (Figures 24 and 25). At 6°C methimazole-fed cockerels have many collapsed follicles and the gland appears as a nearly solid mass of high cuboidal cells (Figure 26). Castrated cockerels fed methimazole have large thyroid follicles that contain considerable amounts of colloid, few reabsorption lacunae and cuboidal follicular cells (Figure 27).

**Pituitary and Thyroid Cyclic AMP-Dependent Protein Kinase Activity During Long and Short Photoperiods**

**Long Photoperiod**

Similar basal cAMP-dependent protein kinase activity ratios were observed in control (0.47 ± 0.01) and castrated cockerels with testosterone implants (0.46 ± 0.02) held at 24°C and exposed to a photoperiod of 14 L:10 D. Seven days after removal of the testosterone implants the pituitary protein kinase activity ratio of castrated birds was elevated
while that of controls remained unchanged. The pituitary activity ratio of the castrated cockerels returned to basal levels (0.51 ± 0.01) two weeks after removal of the testosterone supplements and remained there (0.51 ± 0.02) at 21 days (Figure 28).

The thyroid cAMP-dependent protein kinase activity ratio of castrated cockerels with testosterone implants (0.55 ± 0.02) was identical to that of intact cockerels (0.55 ± 0.03) maintained at the same temperature and photoperiod. After removal of the testosterone supplements, the thyroid activity ratio of these cockerels increased dramatically (0.89 ± 0.01) and remained elevated at 14 (0.76 ± 0.03) and 21 (0.92 ± 0.05) days (Figure 29).

Short Photoperiod

Similar basal pituitary cAMP-dependent protein kinase activity ratios were observed in untreated (0.47 ± 0.01) and testosterone supplemented castrated cockerels (0.48 ± 0.02) maintained at 24 C for 10 weeks on a short lighting schedule (6 L:18 D). After removal of the testosterone implants, the activity ratio was elevated at one week (0.57 ± 0.04), but peak activation (0.84 ± 0.04) occurred at two weeks and remained elevated (0.82 ± 0.02) at 21 days (Figure 28).

The basal cAMP-dependent protein kinase activity ratios were similar in thyroids of untreated (0.58 ± 0.02) and castrated cockerels with testosterone implants (0.55 ± 0.01). Seven days after removal of the testosterone supplements, the thyroid activity ratio was elevated (0.69 ± 0.04) and remained elevated at 14 (0.80 ± 0.05) and 21 (0.80 ± 0.03) days (Figure 29).
Figure 28. Pituitary cAMP-dependent protein kinase activity ratios after removal of testosterone supplements from castrated cockerels that had been maintained for 11 weeks on a long photoperiod (O--O) or 10 weeks on a short photoperiod (●–●). The vertical lines represent ± SEM.
Figure 29. Thyroid cAMP-dependent protein kinase activity ratios after removal of testosterone supplements from castrated cockerels that had been maintained for 11 weeks on a long photoperiod (O---O) or 10 weeks on a short photoperiod (●——●). The vertical lines represent ± SEM.
DISCUSSION

An age-dependent decline in pituitary response to stressful stimuli (149) and low ambient temperature (150) has been previously reported for rats. However, many of the endocrine changes that accompany aging are subtle and detectable only by challenging the responsiveness of the endocrine gland with an injection of its trophic hormone. Using this method an age-related hyporesponsiveness of the mammalian pituitary to TRH has been reported in aged males (151, 152) but not in females (152, 153, 154). The data reported here are believed to be the first indication that White Leghorn chickens exhibit an age-related reduction in the responsiveness of the pituitary-thyroid axis to exogenous TRH.

The administration of TRH dramatically elevates pituitary cAMP-dependent protein kinase activity in both sexes of young birds but does not alter the activity ratio in either sex of aged birds. This reduced responsiveness of the aged pituitary thyrotroph to TRH stimulation implies that hypothalamic regulation of pituitary TSH function is lowered with aging. For example, administration of testosterone propionate to old male rats increases the TSH response to TRH and suggests a close correlation between low serum testosterone and pituitary hyporesponsiveness (151). This, however, does not appear to be valid in the chicken because serum testosterone levels are much greater in two-year-old males than in five-month-old cockerels (155). The mechanisms responsible for
the hyporesponsiveness of the aged avian pituitary to TRH are not known. The effect, however, could be mediated by one or more of the following: a decrease in the thyrotroph population, a reduction in TRH membrane receptors or a failure of the intracellular mechanisms responsible for activating cAMP-dependent protein kinase.

In addition to pituitary failure, a concurrent reduction in thyroid function has been reported with advancing age in mammals (153, 156). As age progresses a reduction in the thyroid hormone secretion rate (157, 158) and plasma \( T_4 \) (151, 153, 156, 159) results in an increase in basal plasma TSH levels (151, 153, 156, 160, 161), presumably as an appropriate thyrotropic response to decreased plasma \( T_4 \). The inability of elevated plasma TSH to increase secretion of thyroid hormone may indicate failure of the thyroid gland to produce hormone. Unfortunately, an assay that accurately measures serum levels of TSH in birds does not exist (162); therefore, an age-dependent increase in plasma TSH cannot be verified for chickens as has been done for mammals (151, 152, 153, 154). However, in chickens of advancing age a reduction in thyroid hormone secretion rate (163, 164, 165) and plasma \( T_4 \) concentrations (112, 166) occurs at the same time as an increase in basal thyroid cAMP-dependent protein kinase activity. These coincidental changes are highly suggestive that an elevation in plasma TSH and a reduction in thyroid gland function also occur in aged chickens.

Injecting TRH into chickens challenges two endocrine functions simultaneously. First, the pituitary is stimulated to produce TSH, and second, the thyroid appears to be capable of responding to either the
direct exogenous stimulation of TRH or endogenous TSH. Since TRH may stimulate the avian thyroid gland (78), the extent of the thyroid protein kinase increase could be dependent upon either the pituitary response to TRH or the direct action of TRH or both. Pituitary TSH secretion appears to be impaired in the old individuals, since TRH fails to increase protein kinase activity. Consequently, it is logical to assume that the thyroid protein kinase response in the aged females is due to the direct action of exogenous TRH and that the thyroid gland of the old males has lost its competence to respond to the neurohormone. In contrast to the situation in aged birds, TRH administration to young individuals stimulates pituitary protein kinase activity. Therefore, the elevated thyroid protein kinase response observed in these birds may be due to either pituitary TSH or exogenous TRH, making interpretation very difficult.

There is no indication of TRH activation of liver or adrenal cAMP-dependent protein kinase in mammalian systems, although rat liver membranes display low-affinity binding for TRH (167). However, chickens injected with TRH increase liver but not adrenal cAMP-dependent protein kinase. Therefore, the activation of liver protein kinase is probably due to either exogenous TRH or endogenous TSH binding to liver membrane receptors, and not increased epinephrine secretion by the adrenal.

The induction of protein kinase activity in the pituitary and thyroid gland by TRH is thought to be a prelude to hormone synthesis and secretion. Previous reports have suggested an elevation in TSH (168, 169) and thyroid hormone secretion (20) subsequent to TRH injection in
Chickens. The injection of TRH induces an extremely rapid elevation in pituitary and thyroid protein kinase activity. Peak pituitary activity is maintained for one hour and then declines rapidly. The rapid decline is correlated with maximum concentrations of plasma $T_3$ and may be due to the negative feedback effects of $T_3$ increasing intrapituitary phosphodiesterase. The thyroid gland maintains maximum protein kinase activity 10 minutes longer than the pituitary but declines gradually and returns to baseline activity after four hours. The slow return to basal level might be due either to the absence of a negative feedback mechanism or to nervous excitement initiated by handling. It was observed that transferring the birds to an unfamiliar surrounding or excessive handling would dramatically increase the basal thyroid protein kinase activity ratio. This effect may be related to adrenergic innervation of the thyroid since norepinephrine containing terminals surround thyroid follicles in several mammalian species (170-172), and the cAMP/cAMP-dependent protein kinase system has been defined as a beta-adrenergic system that may be activated by catecholamines (173). Therefore, a second handling at the time of assay may provide sufficient excitement to prolong the TRH induced increase in thyroid activity.

Baseline concentrations of thyroxine reported here are slightly lower than those previously reported for sexually immature chickens (174-176), but they are similar to those of Japanese quail (177) and much lower than those of mammals (177-179). Subsequent to TRH administration plasma $T_3$ and $T_4$ levels increase in distinctly different temporal patterns with peak levels occurring at 60 ($T_4$) and 80 minutes ($T_3$).
Maximum TRH induced increases in plasma $T_4$ have been reported to take from 30 minutes to four hours in cattle, rats and man (179, 180, 181), while $T_3$ reaches maximum titers in two to four hours in these species (179, 181, 182).

The peak $T_4$ concentrations reported here are much higher than those reported by Klandorf (20) for the Brown Leghorn hen, although both studies used immature hens and both groups were stimulated with 100 $\mu$g injections of TRH. It is possible that the short half-life of TRH and differences in the route of administration will account for the inconsistency. However, even considering these variables, the time required for $T_4$ and $T_3$ to return to basal values is approximately the same in both breeds of chickens but much less than that for mammals (178, 179, 181) or Japanese quail (183). The shorter half-life of $T_3$ and $T_4$ in chickens than in mammals (184) or Japanese quail (185) may account for the apparently more rapid readjustment of hormone levels in White and Brown Leghorn hens (20).

The extrathyroidal conversion of $T_4$ to $T_3$ is probably the primary source of triiodothyronine in birds (13, 20, 174, 186) and mammals (17, 18, 117, 187) and $T_3$ is thought to be the major biologically active hormone (120). The close temporal correlation between the TRH induced increase in plasma $T_4$, plasma $T_3$ and liver thyroxine monodeiodinating activity may indicate a peripheral $T_4$ to $T_3$ conversion in birds. The rise in plasma $T_4$ is rapid, reaching maximum levels at one hour after TRH injection and returning to baseline values 40 minutes later. Maximum liver $T_4$ deiodinating activity occurs at the same time as
thyroxine reaches maximum plasma concentrations and is maintained until \( T_3 \) and \( T_4 \) have returned to baseline levels. The 20-minute time lag between maximum plasma \( T_4 \) and \( T_3 \) suggests an additional time requirement for \( T_3 \) formation. Therefore, the liver of White Leghorn hens appears to contain the necessary processes to deiodinate \( T_4 \) to \( T_3 \). This conversion is probably an enzymatic reaction since TRH administration results in a temporary rise and fall in \( T_4 \) monodeiodinating activity. In addition, the temporal changes in liver thyroxine deiodinating activity may in part account for the cyclic response of plasma \( T_3 \) and \( T_4 \) with the administration of TRH.

Sutherland (188) proposed a major consequence of hormone action to be the regulation of key enzymes in various metabolic pathways. Since cAMP-dependent protein kinase phosphorylates pre-existing enzymes (189, 190, 191), it is possible that the TRH induced increase in liver cAMP-dependent protein kinase activity is responsible for activation of the enzymes involved in the thyroxine deiodinating mechanism. Liver enzyme activation by TRH has not been demonstrated in mammals and this may prove to be another difference in thyroid physiology between mammals and birds.

It is generally assumed that each type of pituitary cell produces a single hormone and once differentiated the cell cannot be converted to a different endocrine function. Conversely, immunocytochemical studies have shown that FSH and LH may occur in the same pituitary cell (192, 193, 194), and pituitary basophils producing LH,
FSH, and TSH may represent a single cell at different stages of its secreting cycle (81, 82).

The pituitaries of castrated cockerels are twice as large as those of control birds but contain the same amount of DNA and protein. The putative gonadotrophs increase by hypertrophy rather than hyperplasia; however, the nature of the cytoplasmic growth is not clear. Protein does not increase during this growth and the cytoplasm of the enlarged cells is difficult to stain. Consequently, it might be speculated that the gonadotroph cytoplasm is composed principally of lipids and/or mucopolysaccharides.

A castration induced increase in adenohypophysial weight has been reported in White Leghorn chickens (195), White Crowned sparrows (196, 197) and the domestic drake (127). The increased weight is believed to be due to the proliferation of gonadotrophs (127). In addition to the effect of castration on pituitary weight, LHRH, photostimulation and gonadal activity may also affect the number, activity and morphology of avian gonadotroph cells (77, 90, 92, 127). Sharp et al. (127), using immunocytochemical and bioassay techniques, observed quantitative changes in the thyrotroph population and TSH levels in castrated and methimazole-fed drakes. The adenohypophysis of methimazole-fed drakes has more TSH/gland than controls and numerous cells that stain with anti-bovine beta TSH. In contrast, the pituitaries of castrated drakes appear to be virtually devoid of TSH. The castration induced increase in adenohypophysial weight of White Leghorns is not associated with a rise in pituitary DNA. This is convincing evidence
that the increased size of the castrated cockerel adenohypophysis is not
due to a mitotic proliferation of gonadotrophic cells. It is suspected
that there is an increase in the number of gonadotrophs and there is
good evidence (127) of a corresponding decrease in the thyrotroph
population.

Thyroxine is indispensable for the normal growth of chickens
(166) and the growth retarding effects of hypothyroidism are evident in
the lower DNA levels of the pituitaries of both groups of methimazole-
fed cockerels. Castrated cockerels fed methimazole have an adeno-
hypophysis the same size as that of controls, while the intact birds
fed methimazole have a pituitary gland significantly smaller than
controls. The pituitary of intact methimazole-fed birds is composed
entirely of small cells while castration plus methimazole treatment
results in a cell population that is morphologically indistinguishable
from that of controls. The small pituitary of methimazole-fed birds is
probably due to an increase in the number of small thyrotrophs and a
decrease in the number of large gonadotrophs. Castration plus methi-
mazole feeding does not alter pituitary DNA, but the treatment does
prevent the disappearance of either cell type.

Average pituitary weight remains constant for each treatment
group even when the animals are exposed to prolonged ambient temperature
extremes (6 C or 33 C). This suggests that ambient temperature may not
be a sufficiently strong stimulus to initiate an alteration in the
thyrotroph population.
Hypothalamic TRH regulation of pituitary TSH secretion in response to cold is well documented in mammals (7, 198, 199), but not in birds. However, TRH has been found in the chicken hypothalamus (200), synthetic TRH is known to influence pituitary-thyroid function (168, 169, 201) and hypothalamic lesions impair TSH secretion and thyroid activity (202, 203, 204) in birds. Cold stress appears to elevate pituitary TSH secretion in both mammals and birds by increasing hypothalamic release of TRH. Low ambient temperature results in pituitary protein kinase responses that are different for each of the treatment groups. Prolonged cold stress (6 C) induces a much greater increase in the pituitary protein kinase activity ratio in methimazole-fed cockerels than in untreated birds and the activity ratio in castrated cockerels, whether fed methimazole or not, is much less than that in untreated birds. This response to cold is thought to be related to the number of pituitary TRH membrane receptors. Methimazole treatment increases the number of thyrotrophs (hence TRH membrane receptors), and castration decreases the number of thyrotrophs.

Sharp et al. (127) has reported that castration has no effect on thyroid weight but may increase plasma thyroxine, reduce pituitary TSH secretion and lower the pituitary thyrotroph population of the domestic drake. Similarly, in the present study there are no substantial differences in weights or spectrochemical determinations between the thyroids of untreated and castrated cockerels. However, cAMP-dependent protein kinase activity is accelerated in the thyroid, but not in the pituitary, of castrated birds. The increased thyroid activity may be
independent of pituitary TSH secretion since the thyroid gland of
castrates did not differ from that of controls. The number of thyro-
trophs is less in castrated cockerels than in untreated birds, and TSH
secretion is presumably depressed by the elevated plasma $T_4$ levels
(127). Consequently, these conditions might be explained by a direct
inhibitory influence of testosterone on the thyroid which is removed
by castration. This supports the findings of Rosenberg (205), who
reports that hypophysectomy decreases body, thyroid, adrenal, comb and
testes weight but does not change thyroid $^{131}$I uptake and increases
thyroid iodine content when expressed per mg of thyroid.

As expected (94), the thyroid glands of methimazole-fed birds
had elevated DNA levels, evident cellular hyperplasia and significantly
increased protein kinase activity ratios. However, castration appears
to moderate the degree of the methimazole induced hypertrophy and hyper-
plasia, as observed by lower DNA levels, larger follicles and more
colloid than in the noncastrated methimazole-fed birds. The mechanism
by which testosterone influences thyroid activity is not known but may
involve selective activation of either type I or type II protein kinase
since each of the forms appears to have a specific biological role
within the cell (40).

Although there is not complete agreement, low ambient tempera-
tures are thought to reduce body weight (206) and increase adrenal and
thyroid weight (206, 207), while high ambient temperatures probably
decrease both body and thyroid weight (208, 209, 210, 211) and increase
adrenal weight (195). Both normal and castrated cockerels have the
greatest body weight gain when maintained at a thermoneutral temperature (24°C) and fed a diet free of goitrogens. The lower body weight of methimazole-fed cockerels is not due to insufficient dietary intake since cockerels fed the standard University of Arizona laying mash and given daily subcutaneous injections of methimazole had body weights as low as those of cockerels receiving the goitrogen in their food. In addition, control cockerels held in ambient temperature extremes (6°C or 33°C) did not have hypertrophied adrenals while castrated birds have enlarged adrenals only when cold stressed (6°C). Since castration may also increase adrenal size (212, 213), the compounding effects of low ambient temperature and castration may be necessary for adrenal hypertrophy.

Hasten and Carmon (208) have reported that thyroid size of White Leghorn cockerels (unlike New Hampshire or White Plymouth Rock cockerels) is not reduced during heat stress and this may be attributed to the higher metabolic rate and greater heat tolerance of White Leghorns. The inverse relationship in the data reported here between thyroid weight and ambient temperature in untreated White Leghorn cockerels clearly contradicts the conclusions of Hasten and Carmon (208). It is reasonable to expect seasonal variation in the size of the thyroid gland since thyroid activity (208, 214, 215) and food intake (216, 217) are greatly reduced during the increased heat of the summer months when compared to winter months.

Unlike intact birds, thyroid weight is not reduced in castrated cockerels during prolonged heat stress although low ambient temperatures
may increase the weight of the gland. The maintenance of thyroid size in castrated birds may be attributed to the removal of the inhibitory action of testosterone on thyroid cAMP-dependent protein kinase activity. Therefore, during periods of high environmental temperature, when cellular activity is normally restricted, the elevated protein kinase activity may stimulate thyroglobulin biosynthesis and colloid formation. In this way castration may prevent acclimation of the thyroid to high environmental temperatures and keep cellular activity artificially high.

High ambient temperature does not reduce thyroid protein kinase activity below the basal 24 C level in untreated birds. There is evidently a minimum level of protein kinase activity that the thyroid gland must maintain and elevated environmental temperatures are not capable of further depression.

Castration and/or methimazole administration combined with any particular ambient temperature has no effect on liver cAMP-dependent protein kinase activity. This was unexpected since castration induces liver fat deposition and lipemia (195) and methimazole causes liver enlargement, deposition of fat and glycogen, proliferation of the intra-hepatic bile ductules and fibrosis (218, 219). It appears that constant exposure to thermal stress may result in an increase in plasma levels of neurogenic amines, epinephrine and norepinephrine (220), which may subsequently increase liver protein kinase activity (221). This elevated activity will remain as long as epinephrine secretion is maintained (222). Also, since no increase in liver protein kinase activity is
observed at 6 C, this temperature is probably not a sufficient stimulus to activate the neurogenic system.

Whether photostimulated or not, castrated cockerels supplemented with testosterone develop their secondary sex characteristics more rapidly than controls, indicating that the implants provide more hormone than the testes of intact cockerels. Serum testosterone levels were not measured; however, Sharp et al. (127) utilized 10 cm implants of the same diameter in castrated drakes and reported serum testosterone levels nine times higher than controls. At the time of assay the testosterone variations did not have any apparent effect on pituitary or thyroid cAMP-dependent protein kinase activity.

The prolonged activation of pituitary protein kinase activity of nonphotostimulated castrates suggests an active hypothalamic-hypophysial regulation of reduced gonadal function in intact photosensitive cockerels subjected to nonstimulatory photoperiods. The small testes of the intact birds appear to inhibit gonadotropic activity, presumably by an inhibitory effect of testosterone on the hypothalamic-hypophysial axis. Testosterone inhibition of pituitary gonadotropin secretion is well established (134, 135, 141, 223, 224) and Cusick and Wilson (225) have demonstrated that testosterone also influences pituitary-gonadal function through feedback on receptors in or near the basal infundibular nucleus of the hypothalamus. Consequently, the reduced LH secretion, depressed growth of the testes and low plasma testosterone levels associated with nonstimulatory photoperiods (133, 136, 139, 226) are probably due to the extreme sensitivity of the
hypothalamus to androgen inhibition allowing the pituitary-gonadal axis to function at a low level.

Pituitary protein kinase activity of 16-week-old photostimulated cockerels increases seven days after removal of the testosterone supplements before dropping to control levels. The failure of these cockerels to maintain an elevated pituitary activity ratio may indicate a weak suppressive effect of high plasma testosterone levels on hypothalamic-pituitary activity in the intact cockerel. The hypothalamic-hypophysial-gonadal axis undergoes its final maturational development at 16 weeks of age (137) and in 18-week-old cockerels castration fails to increase plasma levels. Therefore, the temporary elevation in pituitary protein kinase activity may indicate the final maturational reduction in hypothalamic sensitivity to testosterone inhibition. Regardless of the fluctuations in pituitary response, long day lengths appear to reduce hypothalamic sensitivity to androgen inhibition thus increasing LH secretion, testicular maturation, development of the androgen-dependent accessory organs and expression of sex-related behavior (135, 227). Wilson and Follett (135) maintain that gonadostimulatory effects of long photoperiods are superimposed upon changes in hypothalamic sensitivity.

There is very little information available on the direct effects of photoperiod and gonadal activity on thyroid function in birds. The observations presented here, to some extent, support the evidence compiled for Japanese quail (135, 228) and confirm and extend the findings for photostimulated castrates. Castrated cockerely, whether photostimulated or not, increase thyroid protein kinase activity,
but the extent of the increase is much greater in long day castrates. It is possible that long day lengths may enhance thyroid activity and that their stimulating effects may be superimposed upon the stimulatory effects of reduced plasma testosterone. This supports Peczely et al. (126), who concludes that castration of Japanese quail results in elevated plasma thyroxine levels only when the birds are photostimulated. The presence of a thyroid response to castration in non-photostimulated chickens may suggest that the inhibitory influence of gonadal activity on thyroid function is less sensitive to photoperiod in chickens.

In conclusion, pituitary protein kinase activity increases dramatically following removal of testosterone implants from nonphotostimulated castrated cockerels but drops in photostimulated castrates. Cockerels on short day photoperiods thus appear to maintain their sensitivity to testosterone inhibition of the hypothalamic-pituitary axis, but this sensitivity is lost in photostimulated birds.
REFERENCES


