MASSIE, Ernest Dean, 1944—
THE INTERACTIONS AND INFLUENCES OF THYROXINE
AND DIBUTYRYL 3',5'-ADENOSINE MONOPHOSPHATE
ON TESTICULAR METABOLISM.

The Ohio State University, Ph.D., 1970
Physiology

University Microfilms, A XEROX Company, Ann Arbor, Michigan
THE INTERACTIONS AND INFLUENCES OF
THYROIDINE AND DIBUTYRYL 3', 5'-ADENOSINE
MONOPHOSPHATE ON TESTICULAR METABOLISM

A DISSERTATION

Presented in Partial Fulfillment of the Requirement for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

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The Ohio State University
1970

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ACKNOWLEDGEMENTS

My thanks are due to my adviser, Dr. W. R. Goess for his invaluable guidance and encouragement. Dr. N. L. VanDemark, my department chairman, is deserving of my gratitude for having provided the philosophy and research facilities conducive to productivity.

Mrs. Claudia Jenkins provided excellent technical assistance in the laboratory and artistic talents in the preparation of the graphs contained herein. Mr. Herbert Bridges also provided invaluable technical assistance.

To my wife, Mrs. Karen Massie, I am also grateful for having typed this manuscript and providing constant encouragement.
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INTRODUCTION

The interaction between the thyroid gland and the testis has been described in depth in the literature. The mechanism through which this interaction is mediated still remains unresolved, although a number of physiological, endocrine, and metabolic factors have been implicated. This endeavor was undertaken to examine a specific mechanism through which thyroid hormones may affect male reproduction.

Adenosine 3', 5'-monophosphate (cyclic AMP) is a compound known to mediate a variety of hormonal actions. The literature review contained herein establishes the basis for an intermediation and interaction of cyclic AMP in thyroid hormone effects on testicular metabolic patterns. The purpose of this investigation was to establish and elucidate the interaction between thyroid hormones and cyclic AMP in the metabolic function of testicular tissue. In this project involving 135 albino rats testicular alterations in adenyl cyclase activity, CO₂ production, lipid synthesis, nucleic acid synthesis, and protein synthesis were determined in a variety of endocrine states.
LITERATURE REVIEW

Introduction

Extensive literature indicates a significant relationship between the thyroid and the testes. As the effects of the thyroid gland on male reproduction have recently been reviewed in detail (30, 60), this subject will not be dealt with any further.

The mechanism through which thyroxine affects the testis has remained unresolved. Although different hormones may act through a variety of different mechanisms, one mechanism which has recently been receiving considerable attention is that hormones act through a second messenger. According to this concept the hormones travel to their target tissue where they cause an alteration in the intracellular level of a second messenger. This second messenger then causes cellular changes which are manifest as the physiological expression of the specific hormone. The first such second messenger to have been identified and the one of interest herein is adenosine 3', 5'-monophosphate (cyclic AMP). Cyclic AMP has been identified as a second messenger mediating a variety of hormonal effects (68, 77, 85, 86, 88). To discuss the intermediation by cyclic AMP in thyroidal hormone effects on testicular metabolic patterns, one first needs an understanding of the biological role of the mediator, cyclic AMP.
**Biological Role of Cyclic AMP**

One must first establish the biological role of cyclic AMP before presenting it as an agent acting as the intermediary messenger between thyroxine and the testis. Cyclic AMP was initially reported to be the intracellular mediator of epinephrine and glucagon in the liver (86). The formation of cyclic AMP from adenosine triphosphate (ATP) is catalyzed by an enzyme, adenyl cyclase, usually found in the cell membrane (19,69).

Many hormones are now known to exert some of their effects by altering the intracellular level of cyclic AMP. These hormones act by stimulating adenyl cyclase, which appears to be responsive to different hormones in different tissues. Perhaps, first consideration should be given to a response to cyclic AMP which is fairly well understood. The lipolytic actions of cyclic AMP are one such response.

Lipolysis in the rat epididymal fat pad is stimulated by catecholamines, ACTH, glucagon, TSH, and several other hormones (93). The breakdown of triglycerides to free fatty acids and glycerol provides an insight into the biological role of cyclic AMP, as all the hormones with lipolytic activity have actions mediated by cyclic AMP in tissues with which they are commonly associated. Exogenous cyclic AMP stimulates the release of free fatty acids and glycerol from fat pads and isolated fat cells (4,12,13). As cell membranes are impermeable to cyclic AMP, the dibutyryl derivative has generally been more successful than cyclic AMP in eliciting the lipolytic response.

Krishna et al. (44) found that thyroid hormones also cause a
lipolytic response. They have shown that the mobilization of triglyceride is due to a change in adenylyl cyclase activity rather than an effect on the total lipase activity of the adipose tissue. Fig. 1 shows the mechanism through which the lipolytic response is initiated. Krishna et al. (44) further suggest that the increased adenylyl cyclase activity is due to increased synthesis of the enzyme as the increased activity is blocked by puromycin, a drug that blocks protein synthesis.

![Image of the mechanism of thyroid hormones to lipolysis](image)

**Fig. 1.** Relationship of thyroid hormones to lipolysis.

Fisher and Ball (26) found the mechanism involving mobilization of triglycerides to be less sensitive to lipolytic hormones in hypothyroid and more sensitive in hyperthyroid animals. Brodie et al. (12) confirmed these findings and also reported that adipose tissue from hyperthyroid rats contained a higher amount of epinephrine sensitive adenylyl cyclase. Mandel and Kuehl (56) have also reported that triiodothyronine exerts lipolytic actions by inhibiting phosphodiesterase, the enzyme responsible for the conversion of cyclic AMP to 5'-AMP (Fig. 1).
Besides lipolytic actions, a cyclic AMP mediated steroidogenic response is known to exist in several steroid producing tissues. Perhaps the steroidogenic action of adrenocorticotropic (ACTH) on the adrenal cortex is one of the more intensively studied hormone actions in which cyclic AMP plays a role. The initial experiments demonstrating a cyclic AMP involvement in steroidogenic response to trophic hormones were reported by Haynes and Berthet (35,36), who concluded that cyclic AMP mediated the action of ACTH on the adrenal cortex. Elevated concentrations of cyclic AMP are detectable within 1 minute after the addition of ACTH to adrenal slices, before steroidogenesis initiation, and persist throughout the period of increased steroid production (31). In the absence of ACTH, the addition of cyclic AMP to adrenal slices causes increased steroidogenesis (37). Thus, ACTH appears to act specifically with the adenylyl cyclase system. In addition, Haynes et al. (37) have shown that exogenous cyclic AMP has a steroidogenic effect in vivo, as well as in vitro.

The complete mechanism through which cyclic AMP acts to stimulate steroidogenesis remains unclear. Haynes et al. (38) have shown that cyclic AMP causes phosphorylase activation which leads to increased levels of NADPH (which is required for steroid hydroxylations). Figure 2 illustrates a possible mechanism through which cyclic AMP can influence steroidogenesis.

Unfortunately, evidence indicates that the mechanism of cyclic AMP influence on steroidogenesis is more complex than the mediation of
NADPH in the response. Ferguson (25) found that puromycin inhibited the steroidogenic response of the adrenal to ACTH and cyclic AMP but not to NADPH. Cyclohexamide, another protein synthesis inhibitor, also inhibits the steroidogenic actions of cyclic AMP, but neither puromycin nor cyclohexamide abolished the ACTH stimulation of adeny cyclase (33). This indicates that protein synthesis might be involved in the steroidogenic response, and that phosphorylase activation may not be directly involved, or it may be concomitantly required with protein synthesis.

![Diagram](image)

**Fig. 2** Diagram illustrating the possible mechanism of action of cyclic AMP in steroidogenesis and the site at which inhibitors of protein synthesis may act.
The complete mechanism through which cyclic AMP acts to stimulate steroidogenesis remains unclear. However, evidence has been presented (33,42) which establishes that cyclic AMP affects the steroid synthesis pathway between cholesterol and pregnenolone. It has been postulated that the steroidogenic effect of cyclic AMP is due to a "rapidly turning over protein" (29). However, the nature of its participation, or the mechanism by which its synthesis could be affected by cyclic AMP remains unresolved.

Thyroid hormones also appear to be involved in the steroidogenic response to cyclic AMP. Kowal (43) has reported that thyroxine enhances the stimulatory effect of ACTH on 11 3 hydroxylation, but thyroxine alone has no influence on steroidogenesis or 11 3 hydroxylation in adrenal cell cultures.

Other steroidogenic tissues have also been shown to respond to their trophic hormones through cyclic AMP. Perhaps, the most thoroughly studied of these other tissues has been the action of LH on the bovine corpus luteum. LH increases endogenous cyclic AMP in corpora lutea. This increase in cyclic AMP precedes the LH-induced stimulation of steroidogenesis (58). Marsh and Savard (59) have also reported that the addition of exogenous cyclic AMP mimicked the effect of LH by increasing steroidogenesis, and this effect was not additive to that of LH. It has also been shown (22,57) that the LH increase in cyclic AMP is due to the stimulation adenyl cyclase rather than the inhibition of phosphodiesterase.
The testis is another steroid producing tissue which appears to have a steroidogenic response to cyclic AMP. Sandler and Hall (79) have reported that cyclic AMP increases the conversion of cholesterol to testosterone. LH has been shown (46,66) to increase testicular adenyl cyclase activity. Murad et al. (66) also found that in vitro FSH stimulated adenyl cyclase. However, Kuehl et al. (46) reported that FSH stimulated the adenyl cyclase system only in testes of rats younger than 21 days old.

Evidence also exists which suggests that the steroid influences on their target tissues may also be mediated via cyclic AMP. Szego and Davis (91) have reported that estrogen increases the concentration of cyclic AMP in uteri of ovariectomized rats within 15 seconds. Cyclic AMP also increased RNA synthesis in uteri of ovariectomized rats (82). Further evidence that cyclic AMP is responsible for the physiological actions of estrogen upon the uterus is lacking. However, it is of interest to note that cyclic AMP has been reported to be an inhibitor of ovulation and reproduction (78). These authors suggest that these physiological responses may have been a mimicking of the effects of estrogen.

**Mechanism of Cyclic AMP Action**

Having discussed the biological role of cyclic AMP, one must next consider the manner through which the hormonal responses are mediated. As cyclic AMP elicits a wide variety of physiological responses, numerous possible mechanisms of action for this compound
might be expected. The involvement of cyclic AMP to act at the genetic level to stimulate the synthesis of enzymes will first be discussed.

Enzyme induction requires the transcription of DNA into messenger RNA (mRNA) and the subsequent translation of mRNA into protein. The classic work of Jacob and Monod (41) showed that inducers act at the transcription level to increase enzyme synthesis. Cyclic AMP has been found to stimulate the synthesis of two inducible enzymes, \( \beta \)-galactosidase and tryptophanase (73). Perlman and Pastan (74) also reported that cyclic AMP acts at the level of transcription to increase the synthesis of \( \beta \)-galactosidase mRNA. This work implicated cyclic AMP regulation of \( \beta \)-galactosidase synthesis in mutant \( E. coli \) at the promotor region of the lac operon. Ippen et al. (40) have identified a chromosomal site, the promotor, which controls the rate of \( \beta \)-galactosidase synthesis, and which they postulate to be the point of attachment of RNA polymerase. Further investigations (72) using \( E. coli \) mutants of the lac promoter region, found that these mutants did not respond to cyclic AMP with increased \( \beta \)-galactosidase synthesis. Thus, it has been demonstrated that in \( E. coli \) cyclic AMP interacts with the promotor on the lac operon to regulate the synthesis of a specific enzyme.

In contrast, the regulation of the tryptophanase synthesis by cyclic AMP has been shown (71) to be at the translational rather than the transcriptional level. Cyclic AMP could stimulate enzyme synthesis
at the translational level by either increasing the rate of formation of the polypeptide chains along strands of mRNA or by prolonging the life of the mRNA by preventing its destruction. Evidence (71) indicates that cyclic AMP has no influence on the half life of mRNA. Lissitzky et al. (54) found that cyclic AMP increases the in vitro protein synthesis of thyroid polyribosomes. This lends support to the suggestion that cyclic AMP exerts translational control in higher forms as well as in bacteria.

A third possible mechanism of action for cyclic AMP is the conversion of an inactive enzyme precursor to an active form of the enzyme. Cyclic AMP may exert its very different effects in various tissues by activating specific protein kinases in these tissues. Since Sutherland and Rall (87) first identified cyclic AMP as the mediator of phosphorylase activation by glucagon and epinephrine, a cyclic AMP-dependent protein kinase has been identified as the initial link in a cascading series of protein phosphorylations (95). Cyclic AMP stimulation of casein, protamine, or histone phosphorylation has been observed in enzyme preparations from skeletal muscle (95), liver (48), adipose tissue (17), brain (64), and bacteria (47). The discovery of these cyclic AMP-dependent protein kinases lends support to the hypothesis that the wide variety of effects elicited by cyclic AMP may be mediated through the stimulation of specific protein kinases. The mechanisms through which cyclic AMP stimulates these protein kinases remains unknown. However, it is possible that the
regulation of their synthesis is controlled by cyclic AMP at either
the transcriptional or translational level.

Cyclic AMP is also known to influence the activity of an
enzyme that is already present. Sanwal and Smando (80) have reported
that cyclic AMP is an allosteric inhibitor of malic enzyme in
bacteria. Phosphofructokinase is an enzyme allosterically activated
in the liver fluke (84) and yeast (94).

The discussion of cyclic AMP effects on individual enzyme
systems leads to the consideration of cyclic AMP influences on
metabolism.

**Cyclic AMP Influences on Metabolism**

A discussion of the metabolic effects of cyclic AMP is essential
to prepare the foundation for an investigation of the intermediation
of cyclic AMP in the effects of thyroxine on testicular metabolic
patterns. Cyclic AMP is important in the regulation of metabolism
with its primary influence being on carbohydrate and lipid metabolism.
The metabolic role of cyclic AMP in several tissues is to promote the
mobilization of glucose and fatty acid reserves. Increases in
hepatic cyclic AMP levels result in increased glucose production
through at least three mechanisms: phosphorylase activation,
glycogen synthetase inhibition, and gluconeogenesis stimulation.

The activation of phosphorylase by cyclic AMP has already been
discussed briefly; however, its role is of paramount importance in
considering the influences of cyclic AMP on metabolism. The phos-
phorylase catalyzed reaction is responsible for the conversion of glycogen to glucose. That cyclic AMP mediates the hormonal activation of liver phosphorylase has been known for some time (86). Riley (75) first suggested that cyclic AMP stimulated the activity of dephosphorylase kinase, which in turn activated phosphorylase. Dephosphorylase kinase itself has been found (20,76) to be subject to phosphorylation and dephosphorylation, or activation and inactivation, thus leading to the discovery of the cyclic AMP-dependent protein kinase discussed earlier. Thus the activation of phosphorylase involves a series of reactions with a kinase activating a kinase which in turn activates phosphorylase.

The enzyme which catalyzes the conversion of glucose to glycogen, glycogen synthetase, is also responsive to cyclic AMP. DeWulf and Hers (21) have found that glycogen synthetase could exist in two forms, an inactive phosphorylated form and an active dephosphorylated form. Their evidence suggested that activation was catalyzed by a specific phosphatase while inactivation was controlled by a cyclic AMP-sensitive kinase. Thus, the regulation of glycogen synthetase activity is similar to that of phosphorylase, except that with glycogen synthetase the phosphorylation of the kinase leads ultimately to a decrease in enzyme activity.

Although the effect of cyclic AMP on phosphorylase and glycogen synthetase causes a rapid release of glucose, an effect which is of more importance to the long term maintenance of blood glucose is the
stimulation of gluconeogenesis. Although the enzymatic reactions of gluconeogenesis appear to be known, relatively little is understood about its regulation. Exton and Park (24) have found that the reaction limiting maximum gluconeogenesis is located between pyruvate and phosphoenolpyruvate, and cyclic AMP was found to stimulate this reaction. They have further shown that this stimulation is not due to an inhibition of pyruvate kinase.

It has been suggested (96) that cyclic AMP might increase gluconeogenesis by stimulating hepatic lipolysis. The resulting free fatty acids would be converted to acetyl CoA, which could stimulate pyruvate carboxylase activity. However, this does not seem likely, as Menahan et al. (63) found that acetyl CoA levels did not change in response to glucagon, even though gluconeogenesis was stimulated markedly. This does not rule out the possibility that cyclic AMP could regulate pyruvate carboxylase activity more directly. However, the mechanism by which cyclic AMP regulates gluconeogenesis remains unresolved.

This then leads to a discussion of cyclic AMP effects on lipid metabolism. The lipolytic actions of cyclic AMP have been discussed previously and will not be repeated here. Although the ketone body production by the liver is mostly a function of free fatty acids delivered to the liver through the blood, cyclic AMP stimulates ketogenesis by a direct action within the liver. This may result partly from hepatic lipolysis (9), but mostly from a stimulation
of fatty acid oxidation (39). Heimberg et al. (39) have reported that cyclic AMP, while stimulating fatty acid oxidation, also inhibits triglyceride synthesis.

**Thyroxine–Cyclic AMP Interactions**

Thyroxine influences adenyl cyclase activity in several tissues. The mediation of cyclic AMP in the lipolytic activities of thyroxine have previously been alluded to. Adenyl cyclase activity in adipose tissue is directly responsive to thyroid hormones. Thyroxine increases adenyl cyclase, while thyroidectomy has been shown to decrease the activity of this enzyme to very low values (44). Krishna et al. (44) also reported that puromycin prohibits the stimulation of adenyl cyclase by thyroid hormones. Thus, it appears that thyroxine increases the de novo synthesis of this enzyme, or of an enzyme necessary for increased adenyl cyclase, in fat cells. Challoner (15) has suggested that the stimulation of oxygen consumption by triiodothyronine was mediated through cyclic AMP. Mandel and Kuehl (55) demonstrated an in vitro inhibition of phosphodiesterase by triiodothyronine, but a high level (1.2×10⁻³M) of hormone was required, raising doubt about its physiological significance. Thyroid hormone has also been shown to enhance the lipolytic actions of other hormones including ACTH, TSH, glucagon, and epinephrine (92).

Thyroid hormones have been shown to influence adenyl cyclase activity not only in adipose tissue but also in cardiac tissue. As hypothyroidism causes a decrease in the peak response of myocardial
adenyl cyclase to norepinephrine and fluoride stimulation, Levey et al. (52) have suggested that the total activity of adenyl cyclase per unit of myocardial tissue is depressed in hypothyroidism. Thyroxine has also been reported to stimulate cardiac adenyl cyclase (51). Levey and Epstein (50) have found that maximal stimulatory doses of thyroxine and norepinephrine produce additive effects on cyclic AMP production. This suggests that at least two separate adenyl cyclase systems are present in the heart, one responsive to thyroid hormone and the other to norepinephrine. Caution must be urged, however, as contradictory reports, i.e., that thyroid hormones have no influence on adenyl cyclase activity in the heart, also exist (62,83).

**Thyroxine Effects on Testicular Metabolism**

Thyroxine is most commonly associated with a stimulation of tissue oxygen consumption or metabolic rate. However, the literature indicates that the testis may be one of several tissues that does not respond to thyroxine with an increase in cellular respiration. Barker (7) has reported that thyroxine failed to stimulate oxygen consumption in the rat testis. This is in agreement with previous evidence (8) which suggested that the respiratory rate of testicular tissue did not respond to hyperthyroidism when glucose was used as the exogenous substrate.

The inability of thyroid hormones to stimulate the testicular metabolic rate does not negate the possibility of altered metabolic patterns in testes from animals with different levels of thyroid hormone.
Indeed, previous work (61) has shown that alterations of thyroid status cause glycolytic changes without concomitant respirometric changes when glucose is the exogenous substrate. Hypothyroidism decreased testicular glucose utilization, while hyperthyroidism decreased lactate production.

Thyroxine influence on testicular enzyme systems also deserves consideration. Although the literature contains an abundance of reports on thyroxine interactions with enzyme systems, few reports on thyroxine influences on testicular enzymes exist. Barker (6) found that thyroidectomy lowered succinic dehydrogenase in the testis, but hyperthyroidism failed to increase the activity beyond control levels.

The literature does give some indications that thyroxine has an influence on metabolism in the testis. However, additional research is necessary to clarify and determine specific metabolic alterations in testicular tissue with varying thyroid status. Caution must be exerted in attributing testicular metabolic changes directly to thyroxine as this hormone is known to influence numerous body functions such as growth, maturation, thermoregulation, and basal metabolism. Thus, the effects of thyroxine on testicular metabolism might result from indirect influences of thyroxine on metabolic conditions and homeostasis in the organism as a whole (30).
MATERIALS and METHODS

Treatments

The present study was divided into two categories; one involved the effects of thyroid gland status and cyclic AMP on testicular metabolic patterns, and the other, the effects of thyroxine and gonadotropic hormones on testicular adenyl cyclase activity.

In the metabolic study adult male Wistar rats were randomly assigned to control, hyperthyroid, or hypothyroid groups. Hyperthyroidism was induced by daily subcutaneous injections of 25µg L-thyroxine (Sigma Chemical Company). Hypothyroidism was produced by surgical removal of the thyroid gland (99). The length of the treatment period was 40 days.

Testicular adenyl cyclase was measured in hypophysectomized male Wistar rats (Charles Rivers Laboratory) after treatment with follicle stimulating hormone (FSH), interstitial cell-stimulating hormone (ICSH), or thyroxine. Hypophysectomized and intact control rats were also used. The rats were sacrificed following a 3-day treatment with 100 µg/day of FSH or ICSH, 5 µg/day of thyroxine, or a combination of ICSH and thyroxine; rats were sacrificed 9–18 days after hypophysectomy.

Metabolic Incubations

After the rats were anesthetized with ether, the testes were
removed, trimmed of extraneous tissue, and weighed. The testis
tissue was teased apart with forceps, blotted, weighed, and placed
in radio-respirometric flasks (28). The 100±10 mg portions of tissue
were incubated in 2.6 ml of Krebs-Ringer phosphate buffer (pH 7.4)
containing 300 units of penicillin G. The labeled substrates (Cal-
biochem) for these incubations were either pyruvate-1-14C, pyruvate
-2-14C, glutamate-U-14C, pyruvate-3-14C, or aspartate-U-14C. For
incubations containing the first three of these isotopes a 0.2mM
concentration of dibutyryl 3'-5' adenosine cyclic phosphate
(Calbiochem) was also present. 3.4 umoles of pyruvate and 1 uc
of labeled substrate in a 0.2 ml aliquot were then added to the
media. After a 3 hr. incubation period at 33.5°C, the reaction
was stopped by adding 0.2 ml of 3N perchloric acid. The CO2
was collected in DEAE buffer as described by Free and VanDemark
(28). A liquid scintillation counter was then used to measure the
CO2 collected.

Fractionation
The flask contents were homogenized, then centrifuged. KOH
was added to the supernatant to increase the pH to 11. After
standing 2 hr. at 4°C, the insoluble potassium perchlorate was
centrifuged out. This precipitate was treated as described by
Mounib (65). The precipitate was washed with buffer, then extracted
for lipids (2). The lipid extract was dried, dissolved in scintillation
fluid, and counted. The lipid-free residue was then extracted for
nucleic acids with hot TCA as described by Cohen (16). The residue (protein fraction) was washed with ethanol and ether. The nucleic acid and protein fractions were solubilized with hyamine hydroxide 10X (Packard), suspended in vials, and counted in a scintillation counter.

Adenyl Cyclase Assay

Adenyl cyclase was assayed according to the method of Krishna et al. (45) with modifications for an ATP regenerating system (5) and a different procedure for isolating cyclic AMP (49). The incubation media contained Tris-HCl buffer, pH 7.3 (4x10^{-2}M), MgSO_4 (7.3x10^{-3}M), NaF (1x10^{-2}M), theophylline (1x10^{-2}M), sodium phosphoenolpyruvate (4x10^{-3}M), pyruvate kinase (6x10^{-3}mg, Sigma, Type II) ATP 8^{-14}C (3x10^{-3}M, 0.5 μc), and 10 mg of tissue in a final volume of 0.6 ml. This mixture was incubated at 30°C for 10 minutes. After the addition of 0.1 ml of cyclic AMP (5 mg/ml) as a carrier, the reaction was terminated by immersion for 2-3 minutes in a boiling water bath. The tubes were centrifuged and the supernatant fluid chromatographed on silicic acid impregnated glass-fiber sheets (Gelman, Type SG) (49). Tritiated cyclic AMP (New England Nuclear) standards were run on each sheet to determine the % recovery. The cyclic AMP spots on the sheets were marked under UV light, cut out, and placed into scintillation vials and counted after the addition of 15 ml of standard toluene scintillation medium. Incubations without tissue additions were run as blanks. These blanks were negligible.
RESULTS

Testis and Sex Accessory Gland Weights

As shown in Table 1, testicular wet weights for rats were not altered by hypothyroidism. However, hyperthyroid animals showed a significant decrease ($p < 0.01$) in testis weight. This was partially a reflection of decreased body weights, but a decrease ($p < 0.05$) in the testis weight per 100 grams body weight was still noted.

In the hyperthyroid rats the weight of the seminal vesicle was decreased ($p < 0.01$). There was also a decrease ($p < 0.01$) in the coagulating gland; however, this appeared to be caused by decreased body weight as the coagulating gland weight expressed per 100 grams body weight was not significantly altered. Thus in hyperthyroidism the total sex accessory gland weight was decreased ($p < 0.01$).

Hypothyroidism increased ($p < 0.01$) seminal vesicle weight. However, in the hypothyroid rats there was no alteration in coagulating gland weight. An increase ($p < 0.01$) in total sex accessory gland weight was found when thyroid hormone was lacking.

Nucleic Acids

As illustrated in Fig. 3 and summarized in Table 2, hypothyroidism decreased ($p < 0.01$) in vitro nucleic acid synthesis by rat testis when both pyruvate-$1^{14}C$ and pyruvate-$2^{14}C$ were used as
substrates. As well, exogenous cyclic AMP increased ($p < 0.01$) nucleic acid synthesis from pyruvate-$\text{U}^{14}$C, but not to control values ($p < 0.01$).

When glutamate-$\text{U}^{14}$C and aspartate-$\text{U}^{14}$C were used as substrates, the hyperthyroid condition increased ($p < 0.01$) and $p < 0.05$ respectively) the amino acid incorporation into nucleic acids.

**Lipids**

Thyroid status influenced substrate incorporation into lipids (Fig. 3), mainly when amino acids were utilized. Hyperthyroidism increased ($p < 0.01$) in vitro lipid synthesis when either glutamate-$\text{U}^{14}$C or aspartate-$\text{U}^{14}$C was used. Thyroid hormone also caused an increased ($p < 0.05$) lipid synthesis from pyruvate-$\text{U}^{14}$C. Hypothyroidism increased, but not significantly, the glutamate-$\text{U}^{14}$C incorporation into lipids. However, when exogenous cyclic AMP was added to hypothyroid testicular tissue (Table 3) lipid synthesis from glutamate-$\text{U}^{14}$C was significantly increased ($p < 0.01$) when compared to controls. However, aspartate-$\text{U}^{14}$C incorporation into lipids was also increased ($p < 0.05$) in the thyroidectomized animals.

CO$_2$

As Fig. 3 illustrates, hyperthyroidism decreased ($p < 0.01$) CO$_2$ production from pyruvate-$\text{U}^{14}$C and glutamate-$\text{U}^{14}$C. Cyclic AMP added (Table 4) to the hyperthyroid rat testicular tissue further decreased ($p < 0.01$) glutamate-$\text{U}^{14}$C incorporation into CO$_2$. Hypothyroidism also decreased ($p < 0.01$) CO$_2$ production from glutamate-$\text{U}^{14}$C. However, the CO$_2$ production from pyruvate-$\text{U}^{14}$C increased ($p < 0.05$) in
hypothyroid rat testicular tissue. As shown in Table 4, the addition of cyclic AMP caused a decrease in CO₂ production by control tissue using pyruvate-2-¹⁴C (p<.01) and hypothyroid tissue using glutamate -U-¹⁴C (p<.05).

Proteins

From Fig. 3 it can be noted that hyperthyroidism had no influence on in vitro testicular protein synthesis. However, in the thyroidectomized rats the incorporation of pyruvate-1-¹⁴C (p<.01) and pyruvate-2-¹⁴C (p<.05) into protein (Table 5) was increased.

Adenyl Cyclase

As illustrated in Fig. 4 and summarized in Table 6, neither thyroxine nor FSH treatment altered adenyl cyclase activities from hypophysectomized control values, whereas ICSH injections increased (p<.01) the enzyme activity. However, when ICSH and thyroxine were given in conjunction, adenyl cyclase increased (p<.01) to values comparable to intact control activities. Although testicular weight decreased with time after hypophysectomy, there were no apparent changes in adenyl cyclase activity for any of the groups.
**Table 1**

Effects of the thyroid gland status on testis, sex accessory gland, and body weights.

<table>
<thead>
<tr>
<th></th>
<th>HYPERTHYROID X±SE</th>
<th>CONTROL X±SE</th>
<th>HYPOTHYROID X±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Body Wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g)</td>
<td>296 ± 20</td>
<td>343 ± 24</td>
<td>321 ± 31</td>
</tr>
<tr>
<td>Testis Wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g)</td>
<td>2.78 ± .05</td>
<td>3.45 ± .05</td>
<td>3.62 ± .08</td>
</tr>
<tr>
<td>100g BW</td>
<td>.95 ± .02</td>
<td>1.01 ± .02</td>
<td>1.14 ± .03</td>
</tr>
<tr>
<td>Seminal Ves. Wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg</td>
<td>281 ± 15</td>
<td>415 ± 16</td>
<td>493 ± 22</td>
</tr>
<tr>
<td>mg/100g BW</td>
<td>95 ± 4</td>
<td>121 ± 5</td>
<td>155 ± 7</td>
</tr>
<tr>
<td>Coag. Gland Wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg</td>
<td>79 ± 5</td>
<td>139 ± 7</td>
<td>150 ± 8</td>
</tr>
<tr>
<td>mg/100g BW</td>
<td>27 ± 2</td>
<td>41 ± 8</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Total Acc. Gland Wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg</td>
<td>360 ± 18</td>
<td>553 ± 3</td>
<td>644 ± 24</td>
</tr>
<tr>
<td>mg/100g BW</td>
<td>122 ± 5</td>
<td>162 ± 5</td>
<td>202 ± 9</td>
</tr>
</tbody>
</table>
**TABLE 2**

Influence of thyroid status and exogenous cyclic AMP on *in vitro* nucleic acid synthesis.

<table>
<thead>
<tr>
<th>ISOTOPE</th>
<th>HYPERTHYROID $\bar{X} \pm SE$</th>
<th>CONTROL $\bar{X} \pm SE$</th>
<th>HYPOTHYROID $\bar{X} \pm SE$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate-1-$^{14}$C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.90 ± 0.125</td>
<td>3.92 ± 0.107</td>
<td>3.27 ± 0.098**</td>
</tr>
<tr>
<td>C-AMP</td>
<td>3.95 ± 0.141</td>
<td>3.88 ± 0.114</td>
<td>3.34 ± 0.10**</td>
</tr>
<tr>
<td>Pyruvate-2-$^{14}$C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.00 ± 0.312</td>
<td>12.58 ± 0.37</td>
<td><strong>(9.64 ± 0.248</strong></td>
</tr>
<tr>
<td>C-AMP</td>
<td>12.00 ± 0.343</td>
<td>12.34 ± 0.34</td>
<td><strong>(11.13 ± 0.312</strong></td>
</tr>
<tr>
<td>Glutamate-U-$^{14}$C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.38 ± 0.117**</td>
<td>1.84 ± 0.081</td>
<td>1.95 ± 0.065</td>
</tr>
<tr>
<td>C-AMP</td>
<td>2.32 ± 0.096**</td>
<td>1.83 ± 0.088</td>
<td>1.97 ± 0.084</td>
</tr>
<tr>
<td>Pyruvate-3-$^{14}$C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.77 ± 0.035</td>
<td>.69 ± 0.045</td>
<td>.73 ± 0.041</td>
</tr>
<tr>
<td>Aspartate</td>
<td>2.26 ± 0.032*</td>
<td>1.86 ± 0.126</td>
<td>2.02 ± 0.085</td>
</tr>
</tbody>
</table>

* $\bar{X}$ = mean (+ standard error) recovery of added carbon-$^{14}$

** 1% significance

* 5% significance
### TABLE 3

Thyroid hormone and exogenous cyclic AMP influences on in vitro testicular lipid synthesis

<table>
<thead>
<tr>
<th>ISOTOPE</th>
<th>HYPERTHYROID (X+SE)</th>
<th>CONTROL (X+SE)</th>
<th>HYPOTHYROID (X+SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate-1-14C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>3.21 ± .142</td>
<td>3.06 ± .157</td>
<td>3.13 ± .10</td>
</tr>
<tr>
<td>Pyruvate-2-14C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>8.64 ± .40</td>
<td>7.63 ± .458</td>
<td>8.01 ± .26</td>
</tr>
<tr>
<td>C-AMP</td>
<td>8.69 ± .290</td>
<td>7.79 ± .424</td>
<td>8.63 ± .288</td>
</tr>
<tr>
<td>Glutamate-U-14C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>8.91 ± .259**</td>
<td>6.67 ± .336</td>
<td>7.58 ± .232</td>
</tr>
<tr>
<td>C-AMP</td>
<td>9.01 ± .332**</td>
<td>6.68 ± .349</td>
<td>8.11 ± .252**</td>
</tr>
<tr>
<td>Pyruvate-3-14C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5.95 ± .217*</td>
<td>5.40 ± .173</td>
<td>5.48 ± .117</td>
</tr>
<tr>
<td>Aspartate-U-14C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>6.88 ± .279**</td>
<td>5.29 ± .418</td>
<td>6.35 ± .202</td>
</tr>
</tbody>
</table>

*a* - mean (± standard error) recovery of added carbon -14

** - 1% significance

* - 5% significance
**TABLE 4**

The alteration of CO₂ production in rat testicular tissue by thyroid hormones and exogenous cyclic AMP.

<table>
<thead>
<tr>
<th>ISOTOPE</th>
<th>HYPERTHYROID</th>
<th>CONTROL</th>
<th>HYPOTHYROID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X±SE³</td>
<td>X±SE</td>
<td>X±SE</td>
</tr>
<tr>
<td>Pyruvate—1¹⁴C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.47 ± .623</td>
<td>22.04 ± .885</td>
<td>21.30 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>21.87 ± .562</td>
<td>22.62 ± 1.02</td>
<td>23.45 ± .80</td>
</tr>
<tr>
<td>Pyruvate—2¹⁴C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.84 ± .265**</td>
<td>11.48 ± .356</td>
<td>10.83 ± .34</td>
</tr>
<tr>
<td></td>
<td>9.34 ± .42</td>
<td>10.06 ± .38</td>
<td>10.73 ± .292</td>
</tr>
<tr>
<td>Glutamate—U¹⁴C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>** 9.21 ± .394**</td>
<td>12.29 ± .641</td>
<td>* 8.36 ± .498**</td>
</tr>
<tr>
<td></td>
<td>** 6.91 ± .29**</td>
<td>11.46 ± .511</td>
<td></td>
</tr>
<tr>
<td>Pyruvate—3¹⁴C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.83 ± .374</td>
<td>12.53 ± .453</td>
<td>13.99 ± .408*</td>
</tr>
<tr>
<td>Aspartate—U¹⁴C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.84 ± 1.02</td>
<td>27.72 ± .975</td>
<td>28.23 ± 1.07</td>
</tr>
</tbody>
</table>

a - mean (± standard error) recovery of added carbon —¹⁴
** - 1% significance
* - 5% significance
**TABLE 5**

Protein synthesis as influenced by thyroxine and exogenous cyclic AMP.

<table>
<thead>
<tr>
<th>ISO TOPE</th>
<th>HYPERTHYROID</th>
<th>CONTROL</th>
<th>HYPOTHYROID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X±SE</td>
<td>X±SE</td>
<td>X±SE</td>
</tr>
<tr>
<td>Pyruvate-1(^\text{14C})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>.27 ± .029</td>
<td>.22 ± .018</td>
<td>.36 ± .028(^\text{**})</td>
</tr>
<tr>
<td>C-AMP</td>
<td>.26 ± .02</td>
<td>.25 ± .024</td>
<td>.37 ± .027(^\text{++})</td>
</tr>
<tr>
<td>Pyruvate-2(^\text{14C})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>.57 ± .043</td>
<td>.53 ± .057</td>
<td>.71 ± .049(^*)</td>
</tr>
<tr>
<td>C-AMP</td>
<td>.56 ± .042</td>
<td>.48 ± .043</td>
<td>.74 ± .051(^\text{**})</td>
</tr>
<tr>
<td>Glutamate-U(^\text{14C})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>** (.98 ± .104)</td>
<td>1.06 ± .144</td>
<td>1.33 ± .165</td>
</tr>
<tr>
<td>C-AMP</td>
<td>.95 ± .055(^++)</td>
<td>1.02 ± .099</td>
<td>1.33 ± .188</td>
</tr>
<tr>
<td>Pyruvate-3(^\text{14C})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>1.16 ± .032</td>
<td>1.11 ± .087</td>
<td>1.35 ± .107</td>
</tr>
<tr>
<td>Aspartate-U(^\text{14C})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>2.42 ± .243</td>
<td>2.44 ± .219</td>
<td>2.84 ± .293</td>
</tr>
</tbody>
</table>

\(^a\) - mean (+ standard error) recovery of added carbon \(-\text{14}\)

\(^**\) - 1% significance

\(^*\) - 5% significance
TABLE 6
Testicular weight and adenyl cyclase activity as influenced by gonadotrophins and thyroxine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after hypophysectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9    11  14  16  18  Total</td>
</tr>
<tr>
<td><strong>ICSH</strong></td>
<td></td>
</tr>
<tr>
<td>Ad.c.</td>
<td>469</td>
</tr>
<tr>
<td>T.W.</td>
<td>.85</td>
</tr>
<tr>
<td><strong>FSH</strong></td>
<td></td>
</tr>
<tr>
<td>Ad.c.</td>
<td>143</td>
</tr>
<tr>
<td>T.W.</td>
<td>.79</td>
</tr>
<tr>
<td><strong>T4</strong></td>
<td></td>
</tr>
<tr>
<td>Ad.c.</td>
<td>210</td>
</tr>
<tr>
<td>T.W.</td>
<td>.68</td>
</tr>
<tr>
<td><strong>ICSH T</strong></td>
<td></td>
</tr>
<tr>
<td>Ad.c.</td>
<td>963</td>
</tr>
<tr>
<td>T.W.</td>
<td>.76</td>
</tr>
<tr>
<td><strong>Hypox Con.</strong></td>
<td></td>
</tr>
<tr>
<td>Ad.c.</td>
<td>181</td>
</tr>
<tr>
<td>T.W.</td>
<td>.91</td>
</tr>
<tr>
<td><strong>Intact Con.</strong></td>
<td></td>
</tr>
<tr>
<td>Ad.c.</td>
<td>941</td>
</tr>
<tr>
<td>T.W.</td>
<td>1.24</td>
</tr>
</tbody>
</table>

a - Adenyl cyclase activity in CPM/mg tissue
b - Testis weight in grams
Fig. 3 Influence of thyroid hormone status on metabolic parameters.

Hyperthyroid
Control
Hypothyroid
<table>
<thead>
<tr>
<th>Aspartate-U-C14</th>
<th>Glutamate-U-C14</th>
<th>Pyruvate-3-C14</th>
<th>Pyruvate-2-C14</th>
<th>Pyruvate-1-C14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>CO2</td>
<td>Lipid</td>
<td>Nucleic Acid</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
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<td>5</td>
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<td>6</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

% Recovery/100 mg / 3 hr
Fig. 4 Alteration of testicular adenyl cyclase by gonadotrophins and thyroxine.
HORMONE TREATMENT

- HYPOX
- ICSH
- FSH
- T₄
- ICSH + T₄
- INTACT CONTROL

CPM/mg tissue
DISCUSSION

As shown in this study thyroid hormone does influence testicular metabolism as reflected in alterations of nucleic acid synthesis, lipid synthesis, protein synthesis, and CO$_2$ production. Although thyroxine was found to potentiate the effects of ICSH on adenyl cyclase, cyclic AMP influenced few of the metabolic parameters which were altered by changes in thyroid status.

The influence of thyroid hormone on nucleic acid synthesis (Table 2) is of obvious importance to the exocrine function of the testis. Thyroidectomy resulted in decreased incorporation of the C-1 and C-2 molecules from $^{14}$C-pyruvate into nucleic acids. Neither the incorporation of the C-3 molecule of pyruvate nor the labeled amino acids into nucleic acids was influenced by a decreased level of thyroid hormone. One could suggest that this was due to a decrease in the labeling of the ribose portion of the nucleic acids. The formation of ribose from a pathway to be more carefully examined later is shown in Fig. 5. If there were a decreased production of ribose through this pathway, one would expect a decrease in the CO$_2$ production from C-3. However, hypothyroidism increased CO$_2$ production (Table 4) from C-3, indicating that the rate limiting step in the biosynthetic pathway must be beyond the production of the 5-carbon fragment. Switzer(89) reported the
Fig. 5. Gluconeogenic Pathway
from Pyruvate.
Fig. 6. Precursors of Purines
and Pyrimidines.
PURINE

formate

aspartic acid → N
formate → glycine
formate
amide of glutamine

PYRIMIDINE

carbamyl phosphate

← aspartate
purification of phosphoribosylpyrophosphate (PRPP) synthetase, the enzyme which catalyzes the conversion of ribose 5-phosphate and ATP to 5-phosphoribosyl-1-pyrophosphate. This PRPP synthetase is the first step of a highly branched biosynthetic pathway leading to pyrimidine nucleotides (53) and purine nucleotides (34). Switzer (90) has found that low concentrations of AMP stimulated PRPP synthetase. Thus one might expect cyclic AMP to also stimulate this reaction. In fact dibutylryl cyclic AMP (Table 2) increased the incorporation of C-2 from $^{14}C$-pyruvate into nucleic acids. Ackerman and Al-Mudhaffar (1) found an in vitro stimulation of adenylosuccinate synthetase by thyroid hormones and an inhibition of inosine mono-phosphate dehydrogenase (3). By controlling adenylosuccinate synthetase activity, thyroid hormones could control purine ribonucleotide synthesis. AMP and other purines ribonucleotides inhibit 5-ribosyl-1-pyrophosphate amidotransferase (14,67,97), which catalyzes the first step of purine biosynthesis. Control of AMP synthesis could regulate the supply of adenine nucleotides available for ATP synthesis and nucleic acid synthesis. Cyclic AMP has previously been reported (82) to stimulate RNA synthesis in the ovariectomized rat uterus.

In contrast to thyroidectomy, hyperthyroidism caused an increased incorporation of the amino acids, glutamate-$^1$C and aspartate-$^1$C into nucleic acids. As there was no increase in the carbon atoms from pyruvate, it is suggested that the increase is due to an
increase in the synthesis of purine and pyrimidine bases. Hyperthyroidism has also been reported (11) to cause an increase in the degradation of purines, which might also be indicative of an increased synthesis of these compounds. Fig. 6 illustrates the basic precursors of the purine and pyrimidine skeletons.

Hyperthyroidism also increased the incorporation of amino acids into lipids. As no corresponding increase in pyruvate incorporation into lipids was noted, this might indicate that the high levels of thyroid hormone increase the utilization of amino acids in biosynthetic pathways. Of interest in this regard is also the decrease in CO$_2$ production from glutamate-\textsuperscript{U-14}C and pyruvate-2\textsuperscript{-14}C. It has previously been suggested (60) that the hyperthyroid-induced decrease in testicular oxygen consumption with pyruvate substrate might be due to an increase in amino acid or fatty acid synthesis. As hyperthyroidism had no influence on CO$_2$ production from pyruvate \textsuperscript{-1-14}C, these data suggest that the substrate enters the Krebs cycle, but shunts out of the Krebs cycle (perhaps as fatty acids) at an increased rate.

Conversely, the decrease in CO$_2$ from glutamate-\textsuperscript{U-14}C in hypothyroid testicular tissue may be explained in the opposite manner, i.e., lack of thyroid hormone decreases the utilization of amino acids. However, lipid synthesis from aspartate-\textsuperscript{U-14}C in hypothyroid testicular tissue did increase. Other parameters would indicate a decreased use of Krebs cycle intermediates in biosynthetic pathways.
A decrease in substrate utilization is further supported by previous results (60) which showed decreased glucose utilization and O₂ consumption from pyruvate substrate in hypothyroid testicular tissue.

This investigation also provides results relevant to the metabolism of pyruvate in the rat testis. In all treatment groups the ¹⁴C⁰₂ released from C-1 was greater than that from either C-2 or C-3 (Fig. 3). This is indicative of oxidative decarboxylation of pyruvate through the pyruvate dehydrogenase complex to form acetyl CoA and CO₂. The acetyl CoA may then enter the Krebs cycle by condensing with oxaloacetate or participate in lipid biosynthesis. The CO₂ may be fixed with pyruvate through pyruvate carboxylase to form oxaloacetate which may then be used in the operation of the Krebs cycle, amino acid synthesis, or as a precursor of phosphoenolpyruvate.

Pyruvate carbons entering the Krebs cycle would not include C-1. However, extensive labeling of Krebs cycle intermediates from C-1 of pyruvate, or its equivalent, has been shown (65) to occur. Fleeger et al. (27) demonstrated a stimulatory effect of increasing CO₂ levels on glucose and oxygen uptake, suggesting the importance of pyruvate carboxylation in the rabbit testis.

All the carbon atoms of pyruvate participated in the biosynthesis of lipids, nucleic acids, and proteins. It would thus appear likely that a CO₂ fixation with pyruvate took part in the formation of these fractions. The pyruvate metabolic patterns (Fig. 3) show that C-1 was incorporated into lipids and proteins less than either C-2 or C-3, suggesting that acetyl CoA units from the oxidative
decarboxylation of pyruvate also participated in the biosynthesis of these fractions.

The pattern of incorporation of pyruvate atoms into nucleic acids (Fig. 3) found in this study, is contradicted by Mounib (65), who reported that all atoms were incorporated into nucleic acids equally. However, this was with testicular tissue from cod, a seasonally spawning animal. The ribose fraction of the nucleic acid would contain a greater proportion of C-2 label than either C-1 or C-3. The formation of oxaloacetate through either pyruvate carboxylation or as a Krebs cycle intermediate and subsequent formation of ribose (Fig. 5) could contribute a portion of the C-2 label found in the nucleic acids. However, the labeling pattern of ribose can not account for the very extensive C-2 labeling of nucleic acids (Fig. 3). Perhaps, the high C-2 incorporation into nucleic acids can be explained as due to the formation of formate, which is involved as a precursor of nucleic acid synthesis. When labeled glucose was used as the substrate in incubations of ram spermatozoa (81) and testis tissue from various species (28), formate was labeled to a greater extent than acetate. This might also suggest that the decreased C-2 incorporation into nucleic acids in the hypothyroid testicular tissue was due to a decreased C-2 incorporation into formate.

Another possible entry of C-2 into nucleic acids is through the glycine pool and subsequent incorporation into purines. As
shown in Fig. 6 glycine makes up an integral portion of the purine structure. The hypothyroid induced decrease of C-2 incorporation into nucleic acids (Fig. 3) may be due to either a decrease in the biosynthesis of amino acids or nucleic acids. The inability of hypothyroidism to influence the incorporation of the amino acids, glutamate-U-14C and aspartate-U-14C, into the nucleic acid fraction, suggests that the lack of thyroid hormone alters amino acid biosynthesis.

In this investigation a very low level of incorporation of labeled substrate into proteins was found. This may have been due to the absence of glucose, which has been shown (18) to stimulate the incorporation of lysine-U-14C into testicular protein by 600%. It is also to be noted that the incubation time was only 2 hours.

The addition of dibutyryl cyclic AMP to the incubation media caused a decrease in the incorporation of glutamate-U-14C into protein (Table 5) in hyperthyroid testicular tissue. Halkerston et al. (32) found that theophylline, an inhibitor of the enzymatic transformation of cyclic AMP to 5'-AMP, also decreased the incorporation of labeled amino acids into protein of rat adrenals.

The metabolic parameter mostly frequently influenced by cyclic AMP was CO2 production (Table 4). In all instances in which cyclic AMP exerted an influence, the amount of 14CO2 collected was decreased. This does not necessarily infer a decrease in CO2 production as there may be an increase in fixation of CO2 with pyruvate. Young and Oliver (98) reported dibutyryl cyclic AMP caused a derepression
of pyruvate carboxylase synthesis in the neonatal liver. Thus, if cyclic AMP has the same influence on this enzyme system in the testis, one would find an increase in the CO₂ fixation of pyruvate. The evidence available is compatible with a cyclic AMP-induced gluconeogenesis in the testis. However, before it can conclusively be stated that cyclic AMP is a causative agent of gluconeogenesis in the testis further experimentation is necessary.

Activity of adenyl cyclase, the enzyme responsible for cyclic AMP formation from ATP, in hypophysectomized rat testicular tissue was increased (Fig. 4) by ICSH in vivo. This is in agreement with in vitro ICSH experiments (46,66) which also cause increased adenyl cyclase activity. However, FSH (Fig. 2) did not increase adenyl cyclase activity in the present study, in contrast to the results of Murad et al. (66) who found in vitro FSH to stimulate this enzyme system. Kuehl et al. (46), however, reported that in vitro FSH only stimulates adenyl cyclase in hypophysectomized rats 21 days old or younger. Although thyroxine alone did not affect adenyl cyclase activity, it did enhance the effects of ICSH on testicular adenyl cyclase (Fig. 4). This thyroxine enhancement of a trophic hormone on its target tissue was also found in the case of ACTH influence on 11β hydroxylation in adrenal cells (43).

How does thyroxine potentiate the affects of ICSH on testicular adenyl cyclase? Perhaps when considering thyroxine enhancement of ICSH action on adenyl cyclase, one should also consider the nature
of the site which confers hormone specificity upon a target tissue. The effect of LH on steroidogenesis in interstitial tissue of the rabbit ovary has been shown (23) to be hormone specific. The adenyl cyclase systems in the adrenal (31) and the thyroid (70) have also been shown to be specific for their specific trophic hormones. In this regard it is also of interest to note that thyroxine alone had no influence on testicular adenyl cyclase (Fig. 4). These studies tend to indicate that the activation of adenyl cyclase within a target tissue is a specific property of the hormone that elicits the physiological response in that tissue.

The nature of the site responsible for hormone specificity in a target tissue, the relationship of adenyl cyclase to this site, and the mechanism of activation remain unknown. Studies in different tissues have indicated that the hormonal influence on adenyl cyclase is very rapid, suggesting that adenyl cyclase may be closely associated with the hormone receptor. Thyroxine could increase the affinity of ICSH for its receptor, thus enhancing the effects of the pituitary hormone on adenyl cyclase.

ICSH may also act with its receptor to cause conformation changes in a macromolecular complex of which adenyl cyclase is a part, thus changing the catalytic properties of adenyl cyclase. In this case thyroxine might be capable of accelerating these conformational changes, but be unable to initiate them.

Another possibility to be considered is that the ICSH stimulation
of adenyl cyclase is less direct, involving yet another mediator. If another mediator were involved, the enhancing effects of thyroxine could be accomplished through a direct effect on this other mediator, an increased affinity between ICSH and the mediator, or an increase in the interaction between the mediator and adenyl cyclase.

One must also consider the possibility of at least two testicular adenyl cyclase systems, whose existence is consistent with reports of more than one such enzyme system in various tissues. Bitensky et al. (10) found differences in the biophysical and biochemical properties of the epinephrine and glucagon responsive adenyl cyclase systems in the liver. Levey and Epstein (50) also reported two adenyl cyclase systems in the heart, one responsive to thyroxine and the other to norepinephrine and glucagon. If the two testicular adenyl cyclase systems were independent, one would expect the effects of ICSH and thyroxine on adenyl cyclase to be merely additive. However, the addition of thyroxine to ICSH caused an increase exceeding the additive effects of the two hormones separately. Therefore, either this does not suggest two testicular adenyl cyclase systems, or these systems must be intricately interwoven. If two adenyl cyclase systems do exist in the testis, then the thyroxine stimulated system must first require the stimulation of the other system for the expression of its effects.

Krishna et al. (44) have reported that triiodothyronine increases the de novo synthesis of adenyl cyclase in the epididymal fat pad; if
thyroid hormone causes the same response in the testis, one might also expect an increase in the concentration of adenyl cyclase. However, the enzyme which is synthesized must be in an inactive form as thyroxine alone failed to stimulate adenyl cyclase activity (Fig. 4). If the concentration of inactive adenyl cyclase were increased by thyroxine, more enzyme would be present for ICSH to activate. Thus thyroxine could enhance the effects of this pituitary hormone on testicular adenyl cyclase.

Finally thyroxine may exert its influence on ICSH stimulation of adenyl cyclase through the more general effects of thyroxine on the metabolism of the testicular cells. If thyroxine improves the metabolic state of the cell, an increase in ATP concentration could result. With the resultant increase in substrate the ICSH activated enzyme reaction would proceed at an accelerated rate. If, indeed, thyroxine does enhance ICSH stimulation of testicular adenyl cyclase through an alteration of the metabolic condition of the cells, one would expect thyroxine to enhance the adenyl cyclase activity to target tissue hormones if these tissues are metabolically responsive to thyroxine. This suggestion is supported by Kowal (43) who found thyroxine to enhance the effects of ACTH on some adrenal enzyme systems.

Which, if any, of these suggestions explains the true effect of thyroxine remains unclear at this time. Further purification of the testicular adenyl cyclase system and hormone receptor sites could help to contribute some of these answers.
The overall results of this investigation indicate some general trends between the interactions of thyroxine and cyclic AMP and the metabolic state of testicular tissue. The lack of thyroid hormone tended to decrease the biosynthetic processes with cyclic AMP partially alleviating only the decrease in nucleic acid synthesis from pyruvate-$2\textsuperscript{14}C$. An excess of thyroid hormone tended to increase biosynthesis as reflected in nucleic acid and lipid synthesis. Although thyroxine alone had no influence on testicular adenyl cyclase from hypophysectomized animals, it did enhance the effects of ICSH on this specific enzyme system.
SUMMARY

In an investigation designed to determine the interactions and influences of the thyroid hormone and cyclic AMP on testicular metabolism 135 rats were used with 48 rats being divided into hyper-, hypo-, and euthyroid groups and the testicular tissue utilized to measure in vitro metabolic parameters. Hyperthyroidism was induced by injections of 25 µg day of thyroxine for 40 days. Hypothyroidism was produced by surgical thyroidectomy. The remainder of the rats were either intact controls or hypophysectomized rats used to determine the influence of the endocrine state upon adenyl cyclase activity. The hypophysectomized rats were injected for 3 days with 100 µg FSH/da., 100 µg ICSH/da., 5 µg thyroxine/da., or 100 µg ICSH and 5 µg thyroxine/da.

In hyperthyroid rat testicular tissue, the in vitro incorporation of amino acids into nucleic acid and lipids was increased. Thyroid hormone also increased lipid synthesis from pyruvate-3-14C, while decreasing CO2 production from pyruvate-2-14C and glutamate-U-14C.

While the incorporation of C-1 and C-2 of pyruvate into nucleic acids was decreased, protein synthesis from these isotopes was increased in hypothyroid testicular tissue. Other alterations in in vitro metabolic parameters induced by hypothyroidism included increased lipid synthesis from aspartate-U-14C, increased CO2 production from pyruvate-3-14C, and decreased CO2 production from
Exogenous cyclic AMP increased the incorporation of pyruvate-$C^{14}$ into nucleic acids and glutamate-$U^{14}$C into lipids in the hypothyroid testis. In the hyper- and hypothyroid tissue, cyclic AMP decreased CO$_2$ production from glutamate-$U^{14}$C. The addition of cyclic AMP decreased CO$_2$ production from pyruvate-$C^{14}$ in control tissue.

ICSH increased adenyl cyclase activity from hypophysectomized control values. Although thyroxine alone had no influence, thyroxine given in conjunction with ICSH increased adenyl cyclase activity to values comparable to those from intact controls. FSH was also without influence on testicular adenyl cyclase.
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