INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in “sectioning” the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University Microfilms International
300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD RGW, LONDON WC1R 4EJ, ENGLAND
COMBEST, WENDELL LEE

IMPLICATIONS OF CYCLIC AMP-DEPENDENT PROTEIN KINASES AND POLYAMINE BIOSYNTHESIS IN THE REGULATION OF THE HYPOPHYSIAL-THYROID AXIS.

THE UNIVERSITY OF ARIZONA, PH.D., 1979
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs ✓
2. Colored illustrations
3. Photographs with dark background
4. Illustrations are poor copy
5. Print shows through as there is text on both sides of page
6. Indistinct, broken or small print on several pages throughout
7. Tightly bound copy with print lost in spine
8. Computer printout pages with indistinct print
9. Page(s) lacking when material received, and not available from school or author
10. Page(s) seem to be missing in numbering only as text follows
11. Poor carbon copy
12. Not original copy, several pages with blurred type
13. Appendix pages are poor copy
14. Original copy with light type
15. Curling and wrinkled pages
16. Other
IMPLICATIONS OF CYCLIC AMP-DEPENDENT PROTEIN KINASES AND POLYAMINE BIOSYNTHESIS IN THE REGULATION OF THE HYPOPHYSIAL-THYROID AXIS

by

Wendell Lee Combest

A Dissertation Submitted to the Faculty of the

Department of Pharmacology

through the

COMMITTEE ON PHARMACOLOGY (GRADUATE)

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1979
I hereby recommend that this dissertation prepared under my direction by Wendell Lee Combest entitled IMPLICATIONS OF CYCLIC AMP-DEPENDENT PROTEIN KINASES AND POLYAMINE BIOSYNTHESIS IN THE REGULATION OF THE HYPOPHYSIAL-THYROID AXIS be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Dissertation Director

As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

[Signatures]

Final approval and acceptance of this dissertation is contingent on the candidate's adequate performance and defense thereof at the final oral examination.
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Wendell Combett
To my parents and wife for their patience, support, and understanding. To my mother, who stimulated my interest in biology.
ACKNOWLEDGMENTS

All students owe a great deal to their major advisors for it is through their guidance that they enter the academic community. Dr. Diane Russell has provided this guidance and much more. I would like to thank her for her concern and direction in my development as a scientist. I am especially grateful for her impressing on me the many rewards and excitement of a research career.

I have been privileged to have worked in a stimulating and productive research environment. This is due to the many outstanding people who have been associated with our laboratory from whom I have learned a great deal. I would like to thank Drs. Craig Byus, David Fuller, Jack Olson, and Mari Haddox for their suggestions and advice throughout my graduate study. Dr. Robert Chiasson has been advisor, friend, and collaborator, and without his help the work presented in this dissertation would not have been possible. I am grateful for his interest and patience during the progress of this study.

I would also like to express my appreciation to Dr. Ivan Lytle, Dr. George Hedge, and the members of my graduate committee for their valuable advice and help. In addition, I wish to thank Earl Adams of the Poultry Research Farm for his more than generous help in providing space and facilities for the chicken experiments. I very much appreciate the assistance of Helen Thompson and Dr. Wayne Perris in carrying out the histological studies.
A special note of thanks goes to Missy Mees whose assistance and friendly advice have meant so much to me during the progress of this study. I am grateful to the many others who have given their time and efforts and who have made my graduate education a pleasant and rewarding experience.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Cyclic AMP-Dependent Protein Kinases in Growth and Hormone Action</td>
<td>2</td>
</tr>
<tr>
<td>Polyamine Biosynthesis in Growth and Hormone Action</td>
<td>9</td>
</tr>
<tr>
<td>The Hypophysial-Thyroid Axis</td>
<td>15</td>
</tr>
<tr>
<td>The Adenohypophysis and Thyrotropin-Releasing Hormone (TRH)</td>
<td>17</td>
</tr>
<tr>
<td>The Thyroid Gland and Thyrotropin (TSH)</td>
<td>19</td>
</tr>
<tr>
<td>Thyroid Hormone Action on Peripheral Tissues</td>
<td>21</td>
</tr>
<tr>
<td>Experimental Objectives</td>
<td>23</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>26</td>
</tr>
<tr>
<td>Animal Procedures</td>
<td>26</td>
</tr>
<tr>
<td>Animal Maintenance</td>
<td>26</td>
</tr>
<tr>
<td>Hormone and Drug Administration</td>
<td>26</td>
</tr>
<tr>
<td>Thyroidectomy and Goitrogen Treatment</td>
<td>27</td>
</tr>
<tr>
<td>Ornithine Decarboxylase (ODC) Assay</td>
<td>28</td>
</tr>
<tr>
<td>Kidney Ornithine Decarboxylase Assay</td>
<td>28</td>
</tr>
<tr>
<td>Liver Ornithine Decarboxylase Assay</td>
<td>29</td>
</tr>
<tr>
<td>Adenohypophysial Ornithine Decarboxylase Assay</td>
<td>29</td>
</tr>
<tr>
<td>Thyroid Ornithine Decarboxylase Assay</td>
<td>31</td>
</tr>
<tr>
<td>S-adenosyl-L-methionine Decarboxylase (SAMD) Assay</td>
<td>31</td>
</tr>
<tr>
<td>Cyclic AMP-Dependent Protein Kinase Assay</td>
<td>32</td>
</tr>
<tr>
<td>Liver Cyclic AMP-Dependent Protein Kinase Assay</td>
<td>32</td>
</tr>
<tr>
<td>Thyroid Cyclic AMP-Dependent Protein Kinase Activity</td>
<td>33</td>
</tr>
<tr>
<td>Adenohypophysial Cyclic AMP-Dependent Protein Kinase Activity</td>
<td>34</td>
</tr>
<tr>
<td>Determination of Soluble Type I and II Cyclic AMP-Dependent Protein Kinases</td>
<td>34</td>
</tr>
<tr>
<td>DNA, Protein, Wet and Dry Weight Determinations</td>
<td>36</td>
</tr>
<tr>
<td>Thyroid Histology</td>
<td>37</td>
</tr>
<tr>
<td>Hormone Assays: Thyrotropin (TSH), Triiodothyronine (T₃), and Thyroxine (T₄)</td>
<td>37</td>
</tr>
<tr>
<td>Statistics</td>
<td>38</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS--Continued

RESULTS

<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULTS</td>
<td>39</td>
</tr>
<tr>
<td>Trophic Effects of Thyrotropin (TSH)</td>
<td>39</td>
</tr>
<tr>
<td>Production of Thyroid Growth by Propylthiouracil (PTU) Administration in Rats</td>
<td>39</td>
</tr>
<tr>
<td>Polyamine Biosynthetic Enzymes in Hypertrophy and Hyperplasia of the Rat Thyroid</td>
<td>43</td>
</tr>
<tr>
<td>Cyclic AMP-Dependent Protein Kinases in Rat Thyroid Hypertrophy and Hyperplasia</td>
<td>46</td>
</tr>
<tr>
<td>Responsiveness of the Thyroid Following Hypophysectomy and Chronic Thyrotropin Stimulation in Rats</td>
<td>50</td>
</tr>
<tr>
<td>Chicken Thyroid Hypertrophy During Propylthiouracil (PTU) Treatment</td>
<td>54</td>
</tr>
<tr>
<td>Trophic Effects of Thyrotropin-Releasing Hormone (TRH) and Lowered Plasma Levels of Thyroid Hormone on the Adenohypophysis of the Rat and Chicken</td>
<td>64</td>
</tr>
<tr>
<td>Cyclic AMP-Dependent Protein Kinases in the Adenohypophysis During Propylthiouracil Administration</td>
<td>64</td>
</tr>
<tr>
<td>Effects of Thyroid State on Ornithine Decarboxylase Activity of the Adenohypophysis of the Rat and Chicken</td>
<td>64</td>
</tr>
<tr>
<td>Effects of Triiodothyronine on the Polyamine Biosynthetic Enzymes in Liver and Kidney</td>
<td>73</td>
</tr>
<tr>
<td>Effect of Long-Term Propylthiouracil Treatment on Growth of Various Tissues</td>
<td>73</td>
</tr>
<tr>
<td>Effects of Triiodothyronine and/or Aminophylline on the Activation of Cyclic AMP-Dependent Protein Kinases and the Induction of Ornithine Decarboxylase in Rat Liver</td>
<td>75</td>
</tr>
<tr>
<td>Effects of Somatostatin on the Ability of Triiodothyronine to Induce Ornithine Decarboxylase in Rat Liver</td>
<td>81</td>
</tr>
<tr>
<td>Effects of Inhibitors of RNA and Protein Synthesis on the Activity of Ornithine Decarboxylase after Triiodothyronine or Aminophylline</td>
<td>81</td>
</tr>
<tr>
<td>Triiodothyronine Induction of Kidney Ornithine Decarboxylase</td>
<td>81</td>
</tr>
<tr>
<td>Triiodothyronine Induction of S-Adenosyl-L-Methionine Decarboxylase (SAMD) Activity in Rat Liver</td>
<td>85</td>
</tr>
</tbody>
</table>

DISCUSSION | 88

REFERENCES | 105
<table>
<thead>
<tr>
<th>Figure</th>
<th>Illustration Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polyamine biosynthetic pathway in mammalian tissues</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Time, temperature, and enzyme concentration effects on ornithine decarboxylase activity in the kidney</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Time and enzyme concentration effects on cyclic AMP-dependent protein kinase activity in the rat thyroid</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>Effect of propylthiouracil (PTU) on thyroid wet weight, dry weight, and DNA content/gland in rats</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Histological changes in the rat thyroid following propylthiouracil (PTU) treatment</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>Effect of thyroid stimulating hormone (TSH) administration (1 Unit/100 g BW given i.p.) on the time course of ODC activity in the thyroid of intact rats at various times after administration</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>Effect of propylthiouracil (PTU) on the activities of ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (SAMD) in the thyroid of the rat</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>Effect of administration of propylthiouracil (PTU) on the cyclic AMP-dependent protein kinase activity ratio (-cAMP/+cAMP) in the thyroid of the rat</td>
<td>47</td>
</tr>
<tr>
<td>9</td>
<td>Effect of propylthiouracil administration on the total supernatant activity of cyclic AMP-dependent protein kinase in the thyroid of the rat (measured in the presence of 10 μM cAMP)</td>
<td>48</td>
</tr>
<tr>
<td>10</td>
<td>Elution profile on DEAE-cellulose chromatography columns of cyclic AMP-dependent protein kinases from the rat adenohypophysis (A) and thyroid (B)</td>
<td>49</td>
</tr>
<tr>
<td>11</td>
<td>Effect of propylthiouracil administration on the total amounts of type I and II cyclic AMP-dependent protein kinase in the rat thyroid</td>
<td>51</td>
</tr>
<tr>
<td>12</td>
<td>Effect of hypophysectomy (hypox) on the thyroid ODC response to exogenous TSH</td>
<td>53</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>13</td>
<td>Effect of chronic propylthiouracil stimulation on the ODC response of the thyroid to exogenous TSH in rats</td>
<td>55</td>
</tr>
<tr>
<td>14</td>
<td>Effect of propylthiouracil (PTU) on the thyroid wet weight in chickens</td>
<td>56</td>
</tr>
<tr>
<td>15</td>
<td>Histological changes in the chicken thyroid following propylthiouracil (PTU) treatment</td>
<td>57</td>
</tr>
<tr>
<td>16</td>
<td>Effects of the administration of propylthiouracil on the thyroid cyclic AMP-dependent protein kinase activity ratio (-cAMP/+cAMP) in chickens</td>
<td>60</td>
</tr>
<tr>
<td>17</td>
<td>Effect of propylthiouracil administration on the total supernatant activity of cyclic AMP-dependent protein kinase in chickens</td>
<td>61</td>
</tr>
<tr>
<td>18</td>
<td>Elution profile on DEAE-cellulose chromatography columns of cyclic AMP-dependent protein kinase from the chicken adenohypophysis (A) and thyroid (B)</td>
<td>62</td>
</tr>
<tr>
<td>19</td>
<td>Effect of propylthiouracil administration on the total amounts of type I and II cyclic AMP-dependent protein kinase in the chicken thyroid</td>
<td>63</td>
</tr>
<tr>
<td>20</td>
<td>Effect of propylthiouracil administration on the cyclic AMP-dependent protein kinase activity ratio (-cAMP/+cAMP) of the rat adenohypophysis</td>
<td>65</td>
</tr>
<tr>
<td>21</td>
<td>Ornithine decarboxylase activity (ODC) in the chicken adenohypophysis at various times after thyrotropin-releasing hormone (TRH) administration (0.5 ng/kg i.p.)</td>
<td>67</td>
</tr>
<tr>
<td>22</td>
<td>Effect of different amounts of thyrotropin-releasing hormone (TRH) on ornithine decarboxylase at 4 hr in the rostral (R) and caudal (C) lobes of the adenohypophysis of the chicken</td>
<td>69</td>
</tr>
<tr>
<td>23</td>
<td>Triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) after thyroidectomy and corresponding ornithine decarboxylase (ODC) activity in the adenohypophysis of the rat</td>
<td>71</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS—Continued

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.</td>
<td>Ornithine decarboxylase activity in the rostral (R) and caudal (C) lobes of</td>
<td>72.</td>
</tr>
<tr>
<td></td>
<td>the adenohypophysis of the chicken at 4 hr after thyroxine (T₄) administration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and after methimazole treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>growth of various tissues in the rat</td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>Temporal changes in the activation of cyclic AMP-dependent protein kinase</td>
<td>77.</td>
</tr>
<tr>
<td></td>
<td>and the activity of ornithine decarboxylase (ODC) in rat liver following T₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>administration (300 µg/kg, i.p.)</td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td>Ornithine decarboxylase activity in rat liver 4 hr</td>
<td>78.</td>
</tr>
<tr>
<td></td>
<td>after the administration of various amounts of T₃</td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Time course of activation of cyclic AMP-dependent protein kinase and the</td>
<td>79.</td>
</tr>
<tr>
<td></td>
<td>activity of ornithine decarboxylase in rat liver following aminophylline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>administration (200 µmol/kg, i.p.)</td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>Ornithine decarboxylase activity 4 hr after the injection of triiodothyronine</td>
<td>80.</td>
</tr>
<tr>
<td></td>
<td>(T₃, 300 µg/kg, i.p.), aminophylline (200 µmol/kg, i.p.), or T₃ plus aminophylline in the above doses</td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>The effect of somatostatin pretreatment on the induction of hepatic</td>
<td>82.</td>
</tr>
<tr>
<td></td>
<td>ornithine decarboxylase 4 hr after T₃ administration (300 µg/kg, i.p.)</td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Temporal changes in the activity of ornithine decarboxylase in rat kidney</td>
<td>84.</td>
</tr>
<tr>
<td></td>
<td>following triiodothyronine administration (300 µg/kg, i.p.)</td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>Kidney ornithine decarboxylase activity 4 hr after administration of various</td>
<td>86.</td>
</tr>
<tr>
<td></td>
<td>amounts of triiodothyronine</td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td>Temporal changes in the activity of S-adenosyl-L-methionine decarboxylase</td>
<td>87.</td>
</tr>
<tr>
<td></td>
<td>activity in rat liver following triiodothyronine administration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(300 µg/kg, i.p.)</td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>Proposed model of a general sequence of events in a trophic response</td>
<td>104.</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cyclic AMP-dependent protein kinase type I and type II activities in the rat thyroid following hypophysectomy</td>
<td>52</td>
</tr>
<tr>
<td>2.</td>
<td>Cyclic AMP-dependent protein kinase type I and type II activities in the rat adenohypophysis following propylthiouracil (PTU) treatment</td>
<td>66</td>
</tr>
<tr>
<td>3.</td>
<td>Cyclic AMP-dependent protein kinase type I and type II activities in the chicken adenohypophysis following propylthiouracil (PTU) treatment</td>
<td>66</td>
</tr>
<tr>
<td>4.</td>
<td>Plasma hormone levels and ornithine decarboxylase activity in the adenohypophysis of the rat following TRH administration</td>
<td>70</td>
</tr>
<tr>
<td>5.</td>
<td>Basal ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities in the liver and kidney of rats following a 6-week treatment with propylthiouracil (PTU)</td>
<td>76</td>
</tr>
<tr>
<td>6.</td>
<td>Effect of inhibitors of RNA and protein synthesis on the triiodothyronine stimulation of liver ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities</td>
<td>83</td>
</tr>
</tbody>
</table>
ABSTRACT

The trophic effects of the hormones thyrotropin releasing hormone (TRH), thyrotropin (TSH), and triiodothyronine (T$_3$) on their respective target organs were investigated in rats and chickens. In target tissues, these hormones are capable of inducing ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine biosynthesis, an event which has been shown to be tightly coupled to tissue hypertrophy. Thyrotropin resulted in a 20-fold induction of thyroid ODC by 4 hr in rats. Chronically elevated TSH levels, induced by propylthiouracil (PTU) administration, produced a conspicuous hypertrophy and hyperplasia of the thyroid gland in both rats and chickens.

Ornithine decarboxylase as well as S-adenosyl-L-methionine decarboxylase (SAMD), the enzyme responsible for spermidine synthesis, increased in a biphasic pattern during the progressive glandular hypertrophy. Consistent with the known ability of TSH to elevate cyclic AMP an increased activity ratio of cyclic AMP-dependent protein kinase (-cAMP/+cAMP) in the thyroid occurred during hypertrophy following a pattern similar to fluctuations in ODC and SAMD activities. Changes in cyclic AMP-dependent protein kinase activation generally preceded changes in ODC activity supporting the theory that ODC induction may be a protein kinase mediated event. In addition to an activation of protein kinase, selective changes in the total amounts of the two isozyme types of cyclic AMP-dependent protein kinase were found in the
thyroid following PTU treatment. In both untreated chickens and rats
the thyroid had about 10% type I and 90% type II protein kinase.

Thyroids from hypophysectomized rats were less responsive to
exogenous TSH as evidenced by a reduced ODC response measured at 4 hr
(3-fold as compared to 20-fold elevation in intact animals). A 45%
decrease in the amounts of both type I and type II protein kinase
activity was found in thyroids from hypophysectomized rats which could
explain the lack of effectiveness of TSH to induce ODC. Chronically
stimulated thyroids were shown to respond normally to exogenous TSH.

Thyrotropin releasing hormone (TRH) showed a similar time course
of induction of ODC in the rostral lobe of the chicken adenohypophysis
as that seen with TSH in the rat thyroid. Propylthiouracil treated rats
showed a progressive increase in plasma TSH, elevation of cyclic AMP-
dependent protein kinase activity ratio, and increased ODC activity in
the adenohypophysis. Selective changes in the amounts of type I and
type II protein kinase similar to that observed during thyroid hyper-
trophy occurred in the adenohypophysis during PTU treatment.

Triiodothyronine (T$_3$) and aminophylline resulted in a 6- and
10-fold induction of ODC in rat liver respectively. When both agents
were administered simultaneously, a 16-fold elevation of ODC was
detected indicating an additive effect. Triiodothyronine unlike amin-
ophylline did not produce an activation of cyclic AMP-dependent protein
kinase prior to a detectable increase in ODC activity consistent with
its direct nuclear action. These observations indicate two basic
mechanisms for induction of ODC, one which involves the activation of
cyclic AMP-dependent protein kinase through a cell membrane response and another via a direct nuclear effect.

This study substantiates the induction of ODC as a general and major event in hormone action and tissue hypertrophy. Evidence is presented to further implicate a precise sequence of events involving an increased activation of cyclic AMP-dependent protein kinase and enhanced polyamine biosynthesis as important prerequisites for tissue hypertrophy and hyperplasia. Further studies are necessary to determine the function and importance of alterations in total amounts of type I and type II protein kinases in cell growth processes.
INTRODUCTION

The discovery of 3',5'-adenosine monophosphate (cyclic AMP) in 1957 by the late Dr. Earl Sutherland resulted in the rapid elucidation of the mechanism of action of trophic hormones (1). It was demonstrated for the first time that hormones act at the molecular level and that hormone action could be studied in vitro in disrupted cells with meaningful results. The concept of hormones acting to regulate enzymes in metabolic pathways was introduced and has proven to be a primary way in which hormones exert their intracellular effects. Sutherland introduced the second messenger concept of hormone action where the hormone serves as the first message. The hormonal effects are then translated intracellularly by the second messenger, cyclic AMP.

Today, many intermediate messengers are known to exist, and with the discovery of cyclic AMP-dependent protein kinases and their extensive effect on intracellular metabolism, the concept of a trophic cascade would seem more appropriate. Hormones with direct nuclear actions (steroid and thyroid hormones) as well as membrane-acting polypeptide and amine hormones trigger a sequence of events that occurs in a prescribed temporal order. Some involve cyclic AMP, others clearly do not, in bringing about their observed effects on cellular and tissue function. The present investigation was initiated to test the generality of a "trophic cascade."
The discovery in 1957 of a heat-stable dialyzable factor capable of activating glycogen phosphorylase in broken cell preparations resulted from inquiries into the mechanism of hormone action (1). Epinephrine and glucagon were known to stimulate liver glycogenolysis and the heat-stable factor (cyclic AMP) proved to be the critical link in this process. A revolution related to the possible intracellular role of cyclic AMP occurred in the 1960's at which time many polypeptide and amine hormones were shown to activate membrane adenylate cyclase and increase intracellular cyclic AMP levels. The action of corticotropin (ACTH) on the adrenal cortex, thyrotropin (TSH) on the thyroid, epinephrine on fat cells, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) on the ovary and testes are but a few examples of hormones that were believed to act via a cyclic AMP mechanism (2).

The bewildering diversity of hormone responses supposedly mediated by cyclic AMP was difficult to explain and was responsible for much of the scientific skepticism during these years. It was not until 1968 with the discovery of an enzyme in rabbit skeletal muscle that was activated by cyclic AMP which then transferred the γ-phosphate of ATP to a variety of protein substrates that a mechanism for the diverse actions of cyclic AMP was better understood (3). This enzyme, now referred to as cyclic AMP-dependent protein kinase (ATP:protein phosphotransferase E.C. 2.7.1.37) was first implicated in the cyclic AMP mediation of epinephrine and glucagon stimulated glycogenolysis. Cyclic AMP-dependent protein kinase was demonstrated to phosphorylate phosphorylase b kinase,
which was then active in the conversion of glycogen phosphorylase \( b \) to the active \( a \) form (4). All tissues which have the ability to synthesize cyclic AMP have a demonstrable cyclic AMP-dependent protein kinase and further, hormones previously shown to increase cyclic AMP also activate AMP-dependent protein kinase. These facts led to the postulate that all of the actions of cyclic AMP in mammalian cells were mediated by the activation of cyclic AMP-dependent protein kinase and its ability to alter specific protein phosphorylation patterns (5).

The activation of cyclic AMP-dependent protein kinase by cyclic AMP was extensively studied in the early 1970's (4, 6, 7). Cyclic AMP-dependent protein kinase exists as a relatively inactive holoenzyme \((R_2C_2)\) composed of two inhibiting regulatory subunits \((R_2)\) and two active catalytic subunits \((C_2)\). Cyclic AMP binds to a specific site on the regulatory subunit resulting in a conformational change and subsequent dissociation of the active catalytic subunit. Cyclic AMP-dependent protein kinase catalyzes the transfer of the terminal phosphate of ATP to serine and threonine residues in a variety of enzymes and other proteins found in plasma membranes, mitochondria, and nuclear components. A protein inhibitor \((I)\) first purified from rabbit skeletal muscle and found in many mammalian tissues binds to the catalytic subunits of cyclic AMP-dependent protein kinase and inhibits enzyme activity (8). Other modulatory substances have been described which also could influence the effective protein kinase activity subsequent to its activation by cyclic AMP (9, 10). The biochemical links between activation of protein kinase with accompanying protein phosphorylation and the diverse morphologic and metabolic actions of hormones still are
not well understood. However, recent investigations have implicated cyclic AMP-dependent protein kinases and protein phosphorylation in the regulation of growth processes. Cyclic AMP-dependent protein kinase activity has been shown to change during development in organs such as the lung, heart, liver, brain, and ovary (11, 12, 13) and in regenerating rat liver (14). There is a progressive increase in protein kinase activation as synchronized Chinese hamster ovary cells traverse $G_1$ phase of the cell cycle (15).

Cyclic AMP-dependent protein kinases have been separated into two distinct types by DEAE-cellulose chromatography in a variety of tissues (6, 7, 15-20). Type I elutes from DEAE at a low salt concentration (20-60 mM) whereas type II requires a much higher salt concentration for elution (120-200 mM). Types I and II have markedly different regulatory subunits as evidenced by their differential charge characteristics and migration in SDS polyacrylamide gels, but the catalytic subunits of both types are thought to be identical (21, 22). Recent work has shown that the regulatory subunit of type II protein kinase is autophosphorylated with consequent effects on its ability to reassociate and dissociate (23). Little is known about the physiological roles of the two types of cyclic AMP-dependent protein kinases. Recent studies indicate that the two types of protein kinases are separately regulated and have distinctly separate functions. BALB 3T3 cells have predominantly type II protein kinase. After transformation by simian virus 40, the total amount of type I protein kinase increases (24). The relative amount of type I and II change markedly during the cell cycle of Chinese hamster ovary cells (15), during development in the rat testes (17), and
in various steroid responsive tissues following castration, hypophysectomy, or adrenalectomy (25). Further, the total amount of type I protein kinase activity increases during isoproterenol-induced cardiac hypertrophy (26) and type I is selectively activated early after mitogen stimulation of lymphocyte proliferation (20). Several investigators have suggested that the subcellular localization of the cyclic AMP-dependent protein kinase may play a role in determining the selective activity of the two types of the enzyme (27, 28). Specificity of the protein kinase promoted phosphorylation could be achieved by the enzyme occupying sites adjacent to its protein substrate.

As originally proposed by Sutherland, a major consequence of hormone action is the regulation of key enzymes in various metabolic pathways. This would best explain how a single hormone could so dramatically alter cellular function. Most reports of enzyme regulation by cyclic AMP and cyclic AMP-dependent protein kinase involve a direct phosphorylation of pre-existing enzyme (4, 29-31). Substrates for cyclic AMP-dependent protein kinase have been localized on microsomes and on free and bound ribosomes (16, 32, 33, 34, 35). Although the functional role of ribosome phosphorylation is still unclear, it recently has been reported that the phosphorylation of initiation factors and the 40S ribosomal subunit may play a regulatory role in hemoglobin synthesis (35).

There have been several reports of transcriptional regulation of key enzymes by cyclic AMP (36-38). Perhaps the most extensive evidence for a cyclic AMP mediation of transcription is the induction of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis (39). This is closely related to the function of cyclic AMP as a positive
effector of tissue growth since accumulation of polyamines have been related to increased ribosomal RNA and increased tissue mass (40). The most direct evidence for a cyclic AMP/cyclic AMP-dependent protein kinase-mediated mechanism for ornithine decarboxylase induction is the observation that the ability of many trophic hormones to induce ornithine decarboxylase can be duplicated by cyclic AMP analogues or phosphodiesterase inhibitors. Transcriptional enzyme induction is involved since the increased ornithine decarboxylase activity is blocked by administration of inhibitors of RNA and protein synthesis. Examples of the ability of a cyclic AMP analogue to mimic the trophic hormone induction of ornithine decarboxylase include the following: LH on the ovary and testes (41, 42), TSH on the thyroid (43, 44), ACTH on the adrenal cortex (45), cholinergic stimulation of the adrenal medulla (46) and glucagon and growth hormone on the liver (47, 48). In all of the above-mentioned cases, the induction of ornithine decarboxylase, which usually reaches a maximum by 4-5 hours, is preceded by the activation of cyclic AMP-dependent protein kinase. There is at least a 1-2 hr lag between the maximum activation of cyclic AMP-dependent protein kinase and the maximum ornithine decarboxylase activity, a time lag compatible with a transcriptional event. This same temporal relationship between cyclic AMP-dependent protein kinase activation and ornithine decarboxylase induction is seen in many other growth-stimulated systems, i.e., regenerating rat liver (48), drug-induced liver hypertrophy (49), and adrenal hypertrophy in response to monolateral adrenalectomy (48).

Perhaps the most precise study of such a temporal cascade has been conducted in cell cycle progression studies. Cyclic AMP increases during
the G₁ phase of the cell cycle in both CHV79 cells and in Chinese hamster ovary cells (50-53). Cyclic AMP-dependent protein kinase activation, as well as ornithine decarboxylase activity, increase in a discrete temporal pattern until the G₁/S border when there is a decline in both enzyme activities (50-53). By artificially maintaining an activated protein kinase after cyclic AMP analogue administration, not allowing the decrease normally seen at G₁/S, cells are blocked in G₁ and fail to enter S phase (53).

Another line of evidence linking ornithine decarboxylase induction to the extent of cyclic AMP-dependent protein kinase activation relates to dose response studies of cyclic AMP increases, cyclic AMP-dependent protein kinase activation, and the magnitude of the ornithine decarboxylase response. The phosphodiesterase inhibitors, 3-isobutyl-1-methylxanthine, theophylline, and aminophylline possess differential abilities to elevate intracellular cyclic AMP and to activate protein kinase. This is reflected in their relative ability to induce ornithine decarboxylase (46). Also a positive correlation exists between the ability of 8-substituted cyclic AMP analogues to activate protein kinase in vitro and their ability to increase ornithine decarboxylase activity (54).

The first evidence of the effect of cyclic AMP on a transcriptional process was suggested by Langan (55) in 1966. He observed that either glucagon, which increased cyclic AMP, or dibutyryl-cyclic AMP alone could bring about de novo synthesis of a number of liver enzymes. Correlated with this demonstrated transcriptional effect was the finding that there was a 20-fold increase in the phosphorylation of a specific
serine residue in nuclear F1 histone. This was in line with reports by Kleinsmith and others that suggested a strong positive correlation between phosphorylation of histone and nonhistone chromosomal proteins and growth in various stimulated tissues (56). The phosphorylation appeared to alter the DNA-protein interaction and promote new gene transcription.

It was reported that nuclei isolated from glucagon-stimulated perfused rat liver contained 2 to 3 times as much protein kinase activity as did nuclei from control animals (57). In the presence of either the heat-stable inhibitor or the protein kinase regulatory subunit, the elevation of protein kinase activity in stimulated nuclei was inhibited. This indicated that the increased kinase activity was due to a cyclic AMP-dependent protein kinase. Costa, Kurosawa, and Guidotti (58) in 1976 demonstrated that the tyrosine hydroxylase induction following prolonged stimulation of the adrenal medulla depended on the translocation from cytosol into the nucleus of the low molecular weight catalytic subunit of protein kinase. Dunn-Hunzicker and Jungmann (59) in 1978 demonstrated that the intracellular distribution and enzymatic activity of cyclic AMP-dependent protein kinase in various ovarian structures are subject to regulation by LH or human chorionic gonadotropin. Recently, investigators showed that a protein kinase-dependent phosphorylation occurred in the nuclei of hormone-dependent, 7,12-dimethylbenz[a]-anthracene-induced mammary carcinoma following preincubation of tumor slices with dibutyryl-cyclic AMP (60).

It is therefore likely that one of the consequences of a cyclic AMP-promoted nuclear phosphorylation is the transcriptional control of
the gene for ornithine decarboxylase mRNA synthesis. Thus, in response to a trophic hormone or other growth stimuli, a series of biochemical events are triggered resulting from the initial membrane receptor binding. Elevation of cyclic AMP, activation of cyclic AMP-dependent protein kinase, induction of ornithine decarboxylase, and accumulation of polyamines and ribosomal RNA then occur in a sequential pattern.

**Polyamine Biosynthesis in Growth and Hormone Action**

The naturally occurring polyamines, putrescine, spermidine, and spermine, as well as their biosynthetic enzymes, are found in all life forms from bacteria to plant and animal tissue (61-64). They are the functional and intracellular nonproteinaceous organic cations and indeed, many of their effects, both artifactual and physiological, are attributable to their basic charge. Although present in millimolar concentrations in most cells, they are found in the highest concentrations in cells that are undergoing rapid protein, RNA, and DNA synthesis, i.e., growth.

The polyamine biosynthetic pathway is shown in Figure 1 and involves the decarboxylation of L-ornithine by the enzyme L-ornithine decarboxylase yielding the diamine putrescine (diaminobutane). Subsequent to putrescine formation a second enzyme, S-adenosyl-L-methionine decarboxylase, catalyzes the decarboxylation of S-adenosyl-L-methionine and there is then the transfer of a propylamine moiety from decarboxylated S-adenosyl-L-methionine to putrescine to form spermidine. An additional propylamine group from decarboxylated S-adenosyl-L-methionine
POLYAMINE SYNTHESIS

L-ORNITHINE

ORNITHINE

DECARBOXYLASE

\[
\text{ORNITHINE} \rightarrow \text{NH}_3^+ - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{NH}_3^+
\]

PUTRESCINE

(DIAMINOBUTANE)

\[
\text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{NH}_3^+
\]

from SAM

decarboxylation and propylamine transfer

\[
\text{NH}_3^+ - (\text{CH}_2)_{4} - \text{NH}_3^+ - (\text{CH}_2)_{3} - \text{NH}_3^+
\]

Spermine

from SAM

decarboxylation and propylamine transfer

\[
\text{NH}_3^+ - (\text{CH}_2)_{4} - \text{NH}_3^+ - (\text{CH}_2)_{3} - \text{NH}_3^+
\]

Spermidine

Figure 1. Polyamine biosynthetic pathway in mammalian tissues.
is transferred to spermidine to form spermine which has two primary and
two secondary amine groups.

Many diverse and often confusing observations have been recorded related to the function of polyamines in living organisms. Due to their ubiquitous nature and proclivity to act as nonspecific polycations in \textit{in vitro} experiments, clear-cut physiological roles for polyamines have been slow in developing. However, sufficient evidence has accumulated to strongly implicate polyamines as necessary growth factors. Herbst and Snell (65) as early as 1948 reported that putrescine was a necessary growth factor for the microorganism \textit{Heminophyllus parainfluenza}. This finding has recently been extended to eukaryotes with the finding that proliferation of rat hepatoma cells (HTC) in culture can be blocked by inhibition of ornithine decarboxylase by the competitive inhibitor, $\alpha$-methyl ornithine (66, 67). Removal of the block by addition of ornithine to the culture results in the return of ornithine decarboxylase activity and the resumption of growth. Several additional reports in other mammalian cells in culture indicate the absolute requirement of polyamines for growth (68-72).

Much of the early polyamine research concerned bacterial and viral growth systems. A consistent finding was that polyamines appeared important in maintaining growth and that spermidine concentration was correlated with RNA and protein synthesis. The first studies dealing with the function of the polyamines in vertebrate tissues was carried out by Raina (73, 74) in the early 1960's. His finding along with a similar study by Caldarera, Barbiroli, and Moruzzi (75) indicated a close correlation between increased spermidine and RNA accumulation in
the developing chick embryo. Similar relationships between spermidine and RNA were demonstrated in developing rat brain (76), growth of the sea urchin embryo (77), larval development of the frog (78), and in Drosophila melanogaster (79). A single injection of folic acid in the rat, which destroys kidney tubular cells, stimulates compensatory renal hypertrophy with an accompanying large increase in RNA. The spermidine content of the kidney doubled within 48 hours which closely parallels the net synthesis in RNA (80). In regenerating rat liver, the concentration of spermidine was observed to increase within 24 hours after partial hepatectomy and the rate of spermidine accumulation paralleled the increased rate of RNA synthesis (81, 82). Further, the half-life of spermidine in normal and regenerating rat liver was measured as four days, similar to the half-life for ribosomal RNA in normal liver (83).

Perhaps the most dramatic correlation of spermidine and RNA content was shown by Russell and McVicker (84) in the rat mammary gland during lactation and involution. Maximal milk protein production and secretion occurred coincident with the parallel maximal accumulation of both spermidine and ribosomal RNA. When the mammary gland involuted due to removal of the young from the mother, concentrations of spermidine and ribosomal RNA declined in a parallel manner.

The predominant cellular RNA species is ribosomal RNA which constitutes 70-90% of the total RNA. Ribosomal RNA synthesis in eukaryotes begins with transcription of a 45S ribosomal RNA precursor from the nucleolar rDNA cistrons via RNA polymerase I. Following a complex series of processing steps, 28, 18, and 5.5S rRNA reach the cytoplasm for formation of the ribosomes. The increased activity
of the nucleoli is related to their role in the biosynthesis of the ribosomes; i.e., activation during normal and abnormal growth, and in response to hormones which signal increased macromolecular synthesis (85).

As early as 1960, Cohen and Lichtenstein (86) first reported a significant portion of cellular spermidine present in the ribosomes of E. coli. They were able to demonstrate that spermidine was effective as a complexer of the 30S and 50S subunits to form the normal ribosomal 70S particles. This function of spermidine was similar to the effects of Mg$^{++}$ and led Cohen and Lichtenstein (86, p. 2115) to postulate that "Mg$^{++}$ and sperimidine are the naturally occurring clasps on the complex ribosomal components existing in the bacterium." Raina and Telaranta (87) found that the percentage distribution of spermine and spermidine in a rat liver homogenate resembled that of RNA. Other investigators have shown that the attachment of ribosomes to the total membrane fractions of endoplasmic reticulum occurs only in the presence of 4 mM Mg$^{++}$ or 0.3-0.5 mM spermine (88). It has also been shown that polyamines can block the feedback inhibition of RNA synthesis caused by the addition of exogenous RNA (89). In Xenopus laevis liver cells, it has been demonstrated by autoradiography that $[^3]$Hpolyamines show a similar intracellular accumulation as $[^3]$Huridine with a definite association with the nucleolus (90). These reports and others have led to the belief that polyamines are involved in facilitating ribosomal RNA synthesis and accumulation.

Polyamines also have been postulated as necessary for DNA synthesis and stability. Mandel (91) has shown that exogenous
polyamines increase the resistance of DNA to thermal denaturation. Polyamines complexed to DNA have been isolated from bacteria (92). X-ray crystallographic studies indicate that polyamines may be stereospecifically bound to DNA at the adenine-thymidine linkage and bridge the two polynucleotide strands at the shallow groove of the helix (93). Thus, it is possible that polyamines play a structural role in DNA and may be involved in the complex chromatin changes during cell division. However, there is no definitive evidence in vivo of such an involvement.

Relationships between RNA and polyamines have been investigated by many laboratories. The approach in this laboratory has been to focus attention on the key biosynthetic enzymes, ornithine decarboxylase, and S-adenosyl-L-methionine decarboxylase, which control the polyamine pathway; and on RNA polymerase I related to control of rRNA synthesis.

Evidence linking the parallel accumulation of rRNA and polyamines has been accumulated by Manen and Russell (94) based on extensive data that ornithine decarboxylase in addition to serving as the rate-limiting enzyme in polyamine biosynthesis can serve as an initiation factor for RNA polymerase I, thereby explaining the parallel accumulation of rRNA and polyamines. The most direct evidence supporting this theory is the ability of a near homogeneous preparation of ornithine decarboxylase to increase RNA polymerase I activity in isolated nuclei and nucleoli (94). It has been a remarkably consistent finding that hormones as well as drugs known to stimulate RNA and protein synthesis are capable of inducing ornithine decarboxylase (39). Early studies of the regulation of ornithine decarboxylase activity have revealed that it is regulated transcriptionally and to date it has the shortest half-life of any known
mammalian enzyme (10-20 minutes) (82). Because of its rapid turnover, ornithine decarboxylase activity can fluctuate rapidly in response to the introduction or withdrawal of a stimulus and can be used as a sensitive specific index of increased RNA and protein synthesis.

In order to study the sequence of biochemical events occurring following a growth-promoting stimulus, a well-characterized growth system is needed as a model. In vitro studies have utilized perfused organs, incubated tissue slices, or serum maintained cells in culture as growth models. The advantages of such growth models in terms of decreased variables and simplifications are compromised by the removal from the physiological condition which exists in the intact animal. The present investigation utilizes a well-studied endocrine system, the hypophysial-thyroid axis, as an in vivo model to test the biochemical events related to hormonally stimulated tissue hypertrophy.

The Hypophysial-Thyroid Axis

The term "pituitary or hypophysial-thyroid axis" was first used by Salter in 1930 (cited in 95) and was heralded by Hoskins (96) in 1949 as a classic example of a negative feedback system. Hoskins' (96, p. 1429) description even at this early date is today fundamentally correct: "When the titer of circulating thyroxine rises the anterior pituitary is selectively inhibited and the discharge of thyrotropin is thereby decreased. Contrariwise, episodic or persistent thyroxine deficiency, if sufficient in degree, results in augmented thyrotropin production of more thyroid hormone."
As the above account indicates, the end result is the maintenance of a steady-state plasma level of the thyroid hormones thyroxine ($T_4$) and triiodothyronine ($T_3$). This is accomplished largely by the very precise control of thyrotropin (thyroid-stimulating hormone; TSH) secreting cells in the adenohypophysis known as thyrotrophs. Positive control over the synthesis and release of TSH is exerted by a well-characterized tripeptide of hypothalamic origin, thyrotropin-releasing hormone (TRH) (97, 98). Thyrotropin-releasing hormone is believed to be enzymatically synthesized by neurons localized in principally the paraventricular nuclei of the hypothalamus.

Thyrotropin-releasing hormone is released from nerve endings in the area of the median eminence into a unique plexus of hypothalamic-hypophysial portal blood vessels draining into the sinusoids of the adenohypophysis. Thyrotropin-releasing hormone binds to receptors on the thyrotroph and enhances the synthesis and release of the glycoprotein hormone TSH. Thyrotropin is released into the systemic circulation where it reaches the thyroid gland and binds to specific membrane receptors on the thyroid epithelial cells. Thyrotropin controls essentially all aspects of the incorporation of iodine into the thyroid hormones $T_3$ and $T_4$ as well as their secretion. Triiodothyronine ($T_3$) and/or $T_4$ exert a negative feedback control on the pituitary thyrotroph inhibiting basal as well as TRH-stimulated TSH secretion (99). The hypothalamic neurons producing TRH are influenced by higher brain centers via aminergic neurons principally involving norepinephrine. Recently, somatostatin has been shown to have an inhibitory influence on TRH-stimulated release of TSH (100).
For the purpose of this discussion, the term hypophysial-thyroid axis is extended to include two main target tissues of the thyroid hormones, the liver and kidney. In one respect the metabolic and functional maintenance of these and other organs by a physiological level of plasma thyroid hormone is the true end result of the system. The degradation of the thyroid hormones by the liver, involving the formation of $T_3$ and $T_4$ glucuronide and sulphate conjugates, and subsequent excretion by the kidney are key determinants of the steady-state plasma hormone levels (101). Also, the peripheral deiodination of $T_4$ to $T_3$ by the liver and kidney is important in maintaining adequate amounts of the active form of the hormone, $T_3$ (102).

The Adenohypophysis and Thyrotropin-Releasing Hormone (TRH)

The pituitary or hypophysis in all vertebrates is composed of basically two parts, an anterior portion known as the adenohypophysis arising from a dorsal evagination of the pharynx (Rathke's pouch), and a posterior portion known as the neurohypophysis arising from a ventral development of the floor of the diencephalon (103). The adenohypophysis is a collection of several cell types producing at least 6 main polypeptide hormones under control by different hypothalamic and systemic factors. The TSH producing thyrotrophs make up only a small percentage of the total cells in the anterior pituitary (approximately 10%) in the rat (104). Thyrotrophs are identifiable by their characteristic staining properties, polyhedral shape, and the small size of their TSH-containing granules. The avian adenohypophysis is somewhat different being morphologically divided into rostral and caudal lobes (105).
Radke and Chiasson (106) have found that thyrotrophs responding to circulating thyroid hormones were distributed throughout both lobes of the gland but thyrotrophs responding to TRH were only in the rostral lobe.

It has been well established that both the release and biosynthesis of TSH by the thyrotroph is controlled by hypothalamic TRH. It is also apparent that these effects of TRH are mediated by cyclic AMP and are dependent on Ca\textsuperscript{++} ion (107, 108). Hypothyroid animals have greatly elevated plasma TSH levels and an initial decreased pituitary content of TSH followed by a later recovery due to new TSH biosynthesis. That this thyrotroph response to lowered thyroid hormone levels in hypothyroidism requires TRH has been extensively documented. Lesions of the paraventricular nuclei or hypothalamic deafferentation have been shown to prevent the usual thyroid hypertrophy produced by feeding antithyroid substances (goitrogens) for a 2-week period (109).

In humans with 1° thyroid deficiency of long standing, the weight of the pituitary gland can increase to as much as 150% of normal (103). It has been shown that there is an increase in DNA content, wet weight, and number of pituitary thyrotrophs in rats thyroidectomized for 2 months (104). Many reports note that the thyrotrophs are greatly enlarged and show a more extensive endoplasmic reticulum (103). Again, that TRH is important in this thyrotroph hypertrophy and hyperplasia was suggested by the observation that a single injection of TRH to rats resulted in a marked increase in the number of mitosis in the anterior pituitary 12 hr after injection (110). Therefore, the combined effects of lowered thyroid hormone and stimulation of hypothalamic TRH can lead
to a hypersecretory, hypertrophied, as well as a hyperplastic population of pituitary thyrotrophs.

The Thyroid Gland and Thyrotropin (TSH)

The thyroid gland is present in all vertebrates and is morphologically similar being composed of follicles of epithelial cells surrounding a lumen filled with viscous colloid. Its chief function is to concentrate iodine and to incorporate it into a specialized glycoprotein, thyroglobulin, where it is further utilized in the synthesis of the thyroid hormones $T_3$ and $T_4$. Secretion of the stored $T_3$ and $T_4$ involves a unique process whereby the follicular cell ingests by pinocytosis the lumenal colloid containing the thyroglobulin and thyroid hormones. Following hydrolysis via lysosomal action the free hormones are released into the blood.

All aspects of thyroid hormone synthesis and secretion are regulated by the glycoprotein hormone TSH from the adenohypophysis. Thyrotropin initially interacts with the thyroid follicular cells by binding to specific receptor sites on the plasma membrane (111). These receptors were first demonstrated by Pastan (111) in 1966 and have subsequently been demonstrated in isolated thyroid cells, thyroid slices, homogenates, and purified plasma membranes (112, 113). Subsequent to binding its receptor, TSH activates a membrane localized adenylate cyclase enzyme similar to many other peptide hormones (114). The stimulation of adenylate cyclase leads to increased intracellular cyclic AMP production from ATP. Convincing evidence has now accumulated that cyclic AMP is the intracellular mediator of most if not all of the
actions of TSH on thyroid function. The classical criteria proposed by Sutherland (115) that qualifies cyclic AMP as a mediator of a hormone's action have been satisfied for the thyroid, i.e., (116): (a) TSH has been shown to directly activate thyroid adenylate cyclase in cell-free preparations; (b) TSH promotes the accumulation of cyclic AMP in intact thyroid tissue; (c) the acute and chronic effects of TSH on the thyroid (iodide trapping, iodination of thyroglobin, secretion of $T_3$, $T_4$, and thyroid hypertrophy and hyperplasia) are mimicked by exogenous cyclic AMP and by its analogue, dibutyryl-cyclic AMP; and (d) the effects of low TSH concentrations are potentiated by inhibitors of cyclic AMP phosphodiesterases. Further, cyclic AMP-dependent protein kinases have been found in thyroid tissue that has been activated following TSH or dibutyryl-cyclic AMP administration in vivo and in vitro (117, 118).

Chronic exposure to high levels of TSH as occurs in goitrogen treatment leads to conspicuous hypertrophy and hyperplasia of the thyroid. This involves principally the follicular cells but connective and vascular tissues are also involved (119). The nature of this growth-promoting effect of TSH is revealed in more acute studies where TSH or dibutyryl-cyclic AMP stimulate the incorporation of labeled precursors into protein and RNA (120, 121, 122). Dibutyryl-cyclic AMP as well as TSH increases RNA polymerase I activity of thyroid slices incubated with either agent (121). The effect on protein synthesis appears to involve translational as well as transcriptional mechanisms. The early effect is actinomycin D-insensitive and involves an increased ability of ribosomes to translate message, whereas the latter effect on protein synthesis is completely blocked by actinomycin D (122). Gland
hyperplasia is indicated by an observed increase in cell number, frequency of mitosis, and incorporation of (\(^3\)H)thymidine into DNA (123).

**Thyroid Hormone Action on Peripheral Tissues**

Considerable effort has been focused in the past thirty years on understanding the mechanism of action of the thyroid hormones, triiodothyronine (T\(_3\)) and thyroxine (T\(_4\)), at the cellular level. Two main theories have resulted: (a) thyroid hormones act primarily at the nuclear level to stimulate the transcription of enzymes and other proteins responsible for the observed diverse effects of the hormone; and (b) the primary site of action is within extracellular organelles, i.e., plasma membranes, microsomes, or mitochondria. This theory is based on results of mainly in vitro studies where concentrations of thyroid hormones required to produce the effects are orders of magnitude higher than physiological levels. For this reason, these effects are considered pharmacological and the predominant thinking today is in favor of a nuclear site of action.

The effect of thyroid hormone to increase oxygen consumption and metabolic rate in the whole animal, tissue, as well as in isolated organelles has been recognized for many years, but only recently better understood. Much of the pioneering work was carried out in England by Tata during the early 1960's. Tata's experimental approach involved a series of sequential analyses of biochemical events in hypothyroid rats following a single injection of T\(_3\). The basic finding was that the observed effects on oxygen consumption in liver were preceded by increases in activity of several key enzymes in various metabolic
pathways, i.e., glucose 6-phosphate, α-glycerophosphate dehydrogenase, malic enzyme, and pyruvate carboxylase to name a few (124). The increased activity of these enzymes was blocked by actinomycin D or cycloheximide, indicating de novo enzyme synthesis. One of the earliest events, occurring around 18 hr following a single T₃ injection, was a generalized increase in the incorporation of radiolabeled precursors into protein and RNA. The first enzyme found to increase in activity was RNA polymerase I, the enzyme responsible for ribosomal RNA (rRNA) synthesis, which increased as early as 12 hr. These findings have since been confirmed and extended by several investigators such that the stimulation of nucleolar rRNA with subsequent increase in cytoplasmic ribosomes is a recognized fundamental action of the hormone (125, 126).

A key development in our understanding of thyroid hormone mechanisms has resulted from the work of Oppenheimer in the demonstration and characterization of specific high affinity nuclear binding sites for T₃ (127). They thyroid gland secretes mainly T₄ with T₃, the physiologically active form, being derived principally from peripheral deiodination of T₄. The demonstration of a limited number of T₄ nuclear binding sites by Oppenheimer opens up the possibility of a direct effect of T₄ itself aside from its deiodination to T₃. Studies indicate approximately 5000 T₃ binding sites per nucleus in liver cells. The receptors are associated with the nonhistone chromosomal proteins. The adenohypophysis, liver, and kidney have the greatest number of receptors whereas organs such as the spleen and testes have very few receptors. This is consistent with the observed effects of T₃ on these tissues. Much evidence indicates that a prior interaction of T₃ with a
cytosol receptor is not required for nuclear penetration and binding which is in contrast to the steroid hormones (127, 128). Triiodothyronine appears to interact directly with nuclear receptors to bring about its effects. Consistent with the lack of an initial effect on plasma membranes, $T_3$ appears not to involve the adenylate cyclase cyclic AMP-dependent protein kinase system. However, several reports indicate that enhanced nuclear cyclic AMP-independent phosphorylation takes place following $T_3$ treatment (129-131). Sites of phosphorylation include several specific nonhistone proteins (131), histone $F_1$ (129) and several nucleolar proteins (130). These findings indicate that nuclear kinases via phosphorylation of chromatin-associated proteins could trigger gene expression as has been implicated with other hormones.

**Experimental Objectives**

The hypophysial-thyroid axis and the hormones, TRH, TSH, and $T_3$, provide an excellent model system for studying the hormonal control of tissue hypertrophy and hyperplasia. The physiology of the system is well understood and can be easily manipulated experimentally using appropriate conditions and drugs. Thyrotropin-releasing hormone (TRH) and TSH are broadly representative of the membrane-acting peptides whose diverse actions, both acute and chronic, are generally believed to be mediated via the cyclic AMP-cyclic AMP-dependent protein kinase system. Thus, an understanding of the effects of these hormones on their target organs is likely to be similar to many other cases in which cyclic AMP has been implicated in the control of growth processes.
It is the purpose of this investigation to determine the following:

1. The involvement of cyclic AMP-dependent protein kinases in the trophic responses of the adenohypophysis and thyroid to their respective trophic hormones, TRH and TSH. The activation as well as the total amounts of type I and type II protein kinase were measured in the adenohypophysis and thyroid following propylthiouracil treatment.

2. Because of the large amount of evidence for the involvement of polyamines in growth processes, particularly in the regulation of rRNA, the enzymes controlling polyamine biosynthesis, ODC and SAMD, were studied in relation to TRH and TSH action.

3. Further, the close connection between cyclic AMP-dependent protein kinase activation and polyamine biosynthesis demonstrated in many growth systems was investigated.

4. Triiodothyronine, unlike TRH or TSH, bypasses the membrane-cyclic AMP-cyclic AMP-dependent protein kinase system and elicits its trophic effects by binding directly to nuclear receptors (127). The involvement of polyamine biosynthesis in the action of T₃ on liver and kidney was investigated to contrast with the cyclic AMP-mediated mechanisms of TRH and TSH.

5. The similarity in the growth promoting effects of aminophylline, working via the cyclic AMP axis, and T₃ via an alternate nuclear route, was studied in rat liver.
Results of this investigation indicate a pivotal role for the rate-limiting enzyme of polyamine biosynthesis, ornithine decarboxylase, in hormone action and growth. The enzyme is expressed in a temporally similar manner whether the growth initiating signal is transmitted through receptors at the plasma membrane or acts at receptors in the nucleus.
MATERIALS AND METHODS

Animal Procedures

Animal Maintenance

Sprague-Dawley rats were obtained from the Division of Animal Resources, University of Arizona or purchased from Charles River, Boston, MA. All studies were undertaken using male Sprague-Dawley rats (250-300 gm) unless otherwise indicated. The rats were maintained on a 0700-1900 hr photoperiod and fed ad libitum. Hypophysectomized rats were purchased from Zivic-Miller, Allison Park, PA. Chickens, White Mountain Breeder and White Leghorn hens, were hatched and raised at the University of Arizona Poultry Research Farm. Chickens were housed either in sheltered ground pens or suspended laying cages and fed regular University of Arizona mixed laying mash. Chickens used in this study were provided by the Department of Animal Science, Poultry Research Center.

Hormone and Drug Administration

Triiodothyronine (T₃) and thyroxine (T₄, Sigma Chemical Co., St. Louis, MO) were administered in 0.5 ml of basic saline (0.05 N NaOH and 0.9% NaCl, i.p.). Thyroid-stimulating hormone (thyrotropin, TSH, Sigma) was dissolved in a glucose-phenol solvent (Sigma) and injected in a volume of 0.5 ml of 0.9% NaCl, i.p. Thyrotropin-releasing hormone (pyroglutamyl-histidyl-prolyl-amide, TRH, Calbiochem, Inc., San Diego, CA) was dissolved in 0.5 ml of 0.9% NaCl containing 0.1% BSA and
injected into chickens i.p. or administered i.v. to rats under light ether anesthesia. Somatostatin (Calbiochem, 500 µg/kg, s.c.) was prepared in a protamine-zinc mixture and administered 3 hr prior to T₃ injection. Aminophylline (Sigma, 200 µmol/kg, in 0.9% NaCl) and 3-isobutyl-1-methylxanthine (MIX, Aldrich Chemical Co., Milwaukee, WI; 40 µmol/kg, 0.9% NaCl-ethanol, 5:1, v:v) were administered in a volume of 0.5 ml i.p. Actinomycin D (800 µg/kg, i.p.) and cycloheximide (4 mg/kg, i.p.) were purchased from Calbiochem. All animals were sacrificed by cervical dislocation between 1000-1300 hr to avoid possible diurnal variation in enzyme activities.

Thyroidectomy and Goitrogen Treatment

Surgical thyroidectomy of rats was performed under light ether anesthesia. Addition of 0.2% CaCl₂ in the drinking water following surgery was used to compensate for possible removal of the parathyroids. The high fatality noted after surgical thyroidectomy in chickens makes this procedure impractical. For this reason, chickens were fed 0.1% methimazole (Sigma) in their regular laying mash for 15 days to block thyroid hormone production in one study.

Experimental goiters were produced in rats by the addition of 0.02% 6-n-propyl-2-thiouracil (PTU, Sigma) and 2% sucrose to disguise the taste in their drinking water. Water intake under these conditions was consistent and varied little among animals. The dose of PTU received was approximately 6 mg/rat/day. Goiters were also induced in chickens by addition of either 0.3% (White Mountain Breeders) or 0.1% (White Leghorn) PTU in regular laying mash.
Ornithine Decarboxylase (ODC) Assay

ODC activity was determined by measuring the liberation of $^{14}$CO$_2$ from L-(l-$^{14}$C) ornithine at 37°C for 30 and 60 min as described by Russell and Snyder (82) with minor modifications. Tissue preparation and assay conditions varied slightly between tissues and will be described below. In general the procedure involves tissue homogenization and centrifugation with the resulting supernatant used as the enzyme source. The following homogenization buffer was used for all tissues studied: 0.05 M sodium-potassium phosphate buffer, pH 7.2, containing 0.1 mM EDTA, 1 mM dithiothreitol, and 40 μM pyridoxal phosphate. Buffer was prepared fresh for each experiment from stock solutions stored frozen. Enzyme blanks were determined by the addition to the reaction mixture of 4-bromo-3-hydroxy-benzyloxyamine dihydrogen phosphate (NSD), an inhibitor of pyridoxal phosphate-requiring enzymes, and the value subtracted from all other enzyme determinations. Enzyme activity was assayed in triplicate and expressed as picomoles of $^{14}$CO$_2$ liberated per mg of protein per 30 or 60 min or as percentage of control.

Kidney Ornithine Decarboxylase Assay

Right kidneys were rapidly removed from rats following cervical dislocation and immediately frozen on metal sheets placed over dry ice. Enzyme activity following dry ice freezing was comparable to fresh tissue. The kidney was weighed and homogenized in 5 vol (w/v) of the above mentioned homogenization buffer in a motor-driven glass dual homogenizer (Kontes Glass Co., Berkeley, CA). The homogenates were centrifuged at 40,000 x g for 10 min at 4°C and the resulting
supernatant used as the enzyme source. The reaction mixture included 50 µl of enzyme supernatant (approximately 0.5 mg protein) and 0.5-1.0 µCi of L-(1-14C) ornithine (5 mCi/m mole, New England Nuclear, Boston, MA) in a total volume of 0.2 ml and was assayed for 30 min at 37°C. The reaction was linear for up to at least 1.8 mg protein and 50 min of assay at 37°C. Concentration, temperature, and time linearity studies are presented in Figure 2.

Liver Ornithine Decarboxylase Assay

A small portion of liver always from the same lobe was homogenized in 5 vol of buffer, prepared and assayed as described for kidney. Frozen liver at times gave spurious results possibly resulting from the release of nonspecific decarboxylase activity from the pellet and was therefore avoided. The reaction mixture included 50 µl of enzyme supernatant which represents approximately 1.0 mg of protein. Concentration, temperature, and time linearity for the liver assay as well as the other tissues mentioned was similar to that described for the kidney assay (data not shown).

Adenohypophysial Ornithine Decarboxylase Assay

Chicken adenohypophyses were removed and rapidly separated into rostral and caudal portions on chilled glass slides. The rostral and caudal lobes from two chickens were pooled and homogenized by hand for 15 sec at a moderate rate in 1 ml mini glass homogenizers (prepared from 1 ml Kimax test tubes and glass rods) in 300 and 240 µl respectively of the standard homogenization buffer. Homogenates were centrifuged at 20,000 x g for 15 min at 4°C and the resulting supernatant was used as
Figure 2. Time, temperature, and enzyme concentration effects on ornithine decarboxylase activity in the kidney — (A) Assayed for 30 min at 37°C, (B) assayed for 30 min with 0.9 mg protein/assay, (C) assayed at 37°C with 0.9 mg protein/assay.
the source of enzyme. Single rat adenohypophyses were homogenized as described above in 220 μl of buffer. Assays were the same as described for liver and kidney with the following modifications: the reaction mixture included 90 μl of enzyme supernatant and 1.0 μCi of L-(l-14C)-ornithine (5 mCi/mmmole) in a total volume of 0.1 ml and was assayed for 60 min.

**Thyroid Ornithine Decarboxylase Assay**

Both lobes of the rat thyroid gland were removed and immediately frozen on dry ice. Single glands were weighed, homogenized in 10 vol of the standard buffer, and centrifuged at 20,000 x g at 4°C for 10 min. The reaction mixture contained 50 μl of supernatant (approximately 0.3 mg protein), 0.5 μCi of L-(l-14C)ornithine (10 mCi/mmmole, Amersham Corporation, Arlington Heights, IL), and was incubated for 30 min.

**S-adenosyl-L-methionine Decarboxylase (SAMD) Assay**

S-adenosyl-L-methionine decarboxylase activity was determined by measuring the release of 14CO2 from S-adenosyl-L-(carboxyl-14C)methionine in the presence of 2.5 mM putrescine as the acceptor of the propylamine moiety (132). Tissue preparation, homogenization, and assay conditions for the liver, kidney, and thyroid are identical to that described for the ODC assay. The only difference involved the addition to the assay of 2.5 mM putrescine and 0.5 μCi of S-adenosyl-L-(carboxyl-14C)-methionine (7 mCi/mmmole, New England Nuclear) instead of L-(l-14C)-ornithine. Enzyme activity was assayed in triplicate and expressed as
for ODC in picomoles $^{14}$CO$_2$ liberated per mg protein per 30 min assay at 37°C.

**Cyclic AMP-Dependent Protein Kinase Assay**

Liver Cyclic AMP-Dependent Protein Kinase Assay

Tissue was rapidly removed and homogenized in 10 vol 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 3 min at 10,000 x g in a Beckman Model B microfuge. The resulting supernatant was diluted to 20 vol and 25 µl aliquots were assayed in triplicate for protein kinase activity in the presence and absence of saturating amounts of cyclic AMP (10 µM). Assays were in a total volume of 75 µl of the same buffer with 25 mM magnesium acetate, 200 µg mixed calf thymus histone (Sigma), 0.5-1.0 µCi of (γ-$^{32}$P)ATP (1-5Ci/mmole, New England Nuclear), plus sufficient cold ATP to bring the total substrate concentration to 0.1 mM. The assay was started by addition of the enzyme supernatant and stopped after 5 min at 30°C by spotting 50 µl of the reaction mixture onto Whatman 3 M paper filters. The filters were washed for 20 min in cold 15% trichloroacetic acid (TCA) followed by two additional 15-min washes in 5% TCA, and a 2-min wash in 95% ethanol. The paper filters were air-dried and counted in 5 ml of a toluene-Omnifluor (New England Nuclear) counting fluid. The protein kinase activity ratio (-cAMP/+cAMP) was determined as described by Corbin and Reimann (133) from the amount of $^{32}$PO$_4$ incorporated into acid-insoluble material with and
without cyclic AMP. Enzyme blanks were determined by immediately spotting the reaction mixture.

Thyroid Cyclic AMP-Dependent Protein Kinase Activity

Thyroids from rats or chickens were removed and immediately frozen on dry ice. Tissue was homogenized in 10 vol of the standard homogenization buffer described above for the liver. The homogenate was centrifuged as above and 5 µl (approximately 30 µg protein) of the resulting supernatant assayed for protein kinase activity for 3 min at 30°C. The 5 µl was first pipetted into test tubes containing 45 µl of the homogenization buffer and the reaction initiated by the addition of 25 µl of an assay cocktail resulting in the following final assay concentrations: 20 mM sodium-potassium phosphate, pH 6.5, 0.5 mM 3-isobutyl-1-methylxanthine, 50 µg histone F₁₁b (Sigma), 10 mM magnesium acetate, 5 mM NaF, 0.5-1.0 µCi of (γ-³²P)ATP (5-10 Ci/m mole, New England Nuclear), plus sufficient cold ATP to bring the total concentration to 0.1 mM, and +/- 10 µM cyclic AMP.

Due to the excessive time and tedious washing procedure used with the liver assay, a new method for separating acid-insoluble counts from unreacted (γ-³²P)ATP was employed in the thyroid and adeno-hypophysial assay. The assay was terminated after 3 min at 30°C by pipetting 50 µl of the reaction mixture onto pre-cut strips (1.5 x 5 cm) of Gelman ITLC sheets and chromatographed for 15 min in a tank containing 5% TCA plus 0.2 M KCl. The origin of the strips was pre-spotted with 70 µl of 20% TCA before spotting the 50 µl to insure rapid protein denaturation. The strips were air-dried and the bottom 3 cm portions were cut and
counted in 2 ml of a toluene-Omnifluor (New England Nuclear) counting fluid on a Beckman LS-250 liquid scintillation counter. Enzyme blanks were determined by adding 45 μl of 200 mM EDTA to the standard reaction and subtracting from all other measurements. The EDTA chelates all available Mg$^{++}$ preventing its complexing with ATP to form the proper substrate for the enzyme. The incorporation of $^{32}\text{PO}_4$ into histone was linear for protein concentrations up to approximately 30 μg per assay and for a 5-min incubation at 30°C (Figure 3).

Adenohypophysial Cyclic AMP-Dependent Protein Kinase Activity

Single rat or chicken adenohypophyses were frozen on dry ice and homogenized in 150 μl of the standard homogenizing buffer. Five μl of the 10,000 x g supernatant was assayed as described for thyroid. Assay time and concentration linearity was similar to thyroid.

Determination of Soluble Type I and II Cyclic AMP-Dependent Protein Kinases

Thyroids and adenohypophyses were removed, frozen on dry ice, and stored at -70°C until assay. The glands were homogenized in 10 vol of a 5 mM Tris-HCl buffer, pH 7.5, containing 2 mM NaF and 2 mM EDTA in a motor-driven dual glass homogenizer. Usually 3 thyroids and 5 adenohypophyses were pooled for each determination. The homogenate was centrifuged at 10,000 x g in a Beckman Model B microfuge for 5 min at 4°C and 0.5 ml of the supernatant applied to a 10 x 1 cm DEAE-cellulose column (Whatman DE-52). A small aliquot of the supernatant applied to the column was assayed for protein kinase activity. The column was washed with 15 ml of the above buffer prior to initiation of a 30-ml
Figure 3. Time and enzyme concentration effects on cyclic AMP-dependent protein kinase activity in the rat thyroid—Assayed in the presence (o--o) or absence (o--o) of cyclic AMP.
(15 ml in each beaker) linear salt gradient from 0 to 400 mM NaCl in the same washing buffer. Thirty 1 ml fractions were collected by a Gilson fraction collector and a 50 µl aliquot of each fraction assayed for protein kinase activity in the presence of cyclic AMP (10 µM) as described earlier. The column flow rate was approximately 20 ml/hr. Under these conditions there was complete reassociation of the protein kinase subunits to the holoenzyme form. No protein kinase activity was detected in the flow through during washing.

Two peaks of cyclic AMP-dependent protein kinase activity were found in both the thyroid and adenohypophysis corresponding to type I and II found in many other tissues. Conductivity measurements of the salt gradient fractions indicate that type I and type II cyclic AMP-dependent protein kinase elute at 20-60 mM and 120-200 mM osmolarity, respectively. The amounts of type I and II were determined by totalling the activity under each peak and expressed as pmoles of $^{32}$PO$_4$ incorporated per min per mg protein added to the column. Recovery from the column was consistent and was approximately 80%.

**DNA, Protein, Wet and Dry Weight Determinations**

DNA in thyroid tissue was determined by a modified fluorometric method described by Setaro and Morley (134). The quantitation involves the reaction of 3,5 diaminobenzoic acid dihydrochloride (DABA, Aldrich Chemical Co.) with deoxyribose liberated from DNA by acid hydrolysis. Fluorescence was measured by an Aminco-Bowman Spectrofluorometer (excitation 420 nM, emission 520 nM using calf thymus DNA [Sigma] as standard).
Protein was determined either by the method of Lowry et al. (135) or Bradford (136) using bovine serum albumin as standard. Thyroid wet weight was determined using an Ainsworth balance. Thyroid dry weight was determined after 12 hr in a drying oven at 50°C.

**Thyroid Histology**

Thyroid glands were fixed in 10% formalin and embedded in paraffin blocks. Samples were sectioned (5μ) and dried for 1 hr in a 66°C drying oven and treated with a series of xylene and alcohol washes before staining with hematoxylin and eosin. Slides were microscopically examined (400 x enlargement) and photographed using Kodak Tri-X black and white film and printed on Agfa-Gevaert brovira BS 1 paper.

**Hormone Assays: Thyrotropin (TSH), Triiodothyronine (T₃), and Thyroxine (T₄)**

All hormone measurements were performed in the laboratory of Dr. George Hedge, Department of Physiology, in conjunction with a collaborative experiment. Thyrotropin was assayed using radioimmunoassay materials provided by the NIA MDD Rat Pituitary Hormone Distribution Program, and the data are expressed as micrograms of rat TSH per 100 ml of plasma using NIA MDD rat TSH-RP-1 (0.22 U/mg) as standard. Carrier-free ^{125}I for iodination of TSH and for determination of thyroid iodine release was purchased from ICN Pharmaceuticals, Inc., Irvine CA. Specific radioimmunoassays for T₃, Chopra, Ho, and Lam (137), and T₄, Chopra (138), were performed using antisera provided by Dr. D. A. Fisher (Harbor General Hospital, Torrance, CA). The radioimmunoassay of T₃ (as described for human serum) was modified for use with rat plasma by
substituting $T_3$-free rat plasma (instead of $T_3$-free sheep serum) in all tubes which did not already contain rat plasma. $T_3$-free rat plasma was prepared by charcoal extraction according to the method of Larsen (139). The assay was performed on 250 µl of plasma, and the hormone was displaced from binding proteins by adding 250 µg of 8-anilino-1-napthalene sulfonic acid (ANS) to each tube.

Statistics

All data were analyzed by the Student t test for significance unless otherwise stated.
RESULTS

Trophic Effects of Thyrotropin (TSH)

Production of Thyroid Growth by Propylthiouracil (PTU) Administration in Rats

Administration of 0.02% PTU for 14 days in the drinking water of rats resulted in a marked enlargement of the thyroid gland with wet and dry weight, and DNA/gland increasing to 280%, 270%, and 240% of control, respectively (Figure 4). Thyroid wet weight increased to 120% of control (p < 0.01) as early as one day following the initiation of PTU treatment. By two days, the dry weight had increased significantly, an indication that the increased weight was due to an increase in tissue mass rather than edematous water accumulation or blood engorgement. There appear to be two general stages of growth during the 14-day test period; an initial hypertrophy phase where an increase in thyroid weight is due primarily to cell enlargement (0-3 days) and a second stage starting between 3 and 4 days where cells apparently initiate DNA synthesis and divide. A steep increment of thyroid growth was observed between day 2 and 3 followed by a cessation of weight gain between day 3 and 4 coincident with the rise in DNA content.

Marked changes in thyroid histology were apparent during the progressive stages of gland hypertrophy and hyperplasia (Figure 5). Thyroid epithelial cells, after only 2 days of PTU treatment, were enlarged and columnar in appearance compared to the flattened or cuboidal cells characteristic of the unstimulated basal state. A
Figure 4. Effect of propylthiouracil (PTU) on thyroid wet weight, dry weight, and DNA content/gland in rats — Rats were given 0.02% PTU in their drinking water for various time periods. Each animal received approximately 6 mg of PTU per day. Data are expressed as the mean ± S.E.M. of at least 6 rats.
Figure 5. Histological changes in the rat thyroid following propyl-thiouracil (PTU) treatment — Glands were stained by a standard hematoxylin and eosin procedure and magnified 400 X. (A) Control, (B) 2 days of PTU treatment, (C) 4 days of PTU treatment, (D) 14 days of PTU treatment.
Figure 5.—Continued
decrease in follicular colloid content was also seen after 2 days indicating enhanced thyroid hormone secretion involving endocytosis and lysosomal digestion of the colloid. The columnar cells at 2 days had conspicuously enlarged nuclei but showed no signs of cell division. By 4 days cells were still enlarged, little follicular colloid remained, and there was evidence of increased cell number. By 14 days, the thyroid appeared quite different from the normal state with little evidence of a follicular structure. No colloid was present and marked hyperplasia was apparent.

Polyamine Biosynthetic Enzymes in Hypertrophy and Hyperplasia of the Rat Thyroid

A single injection of TSH (1 unit/100 g BW i.p.) resulted in a 20-fold elevation of thyroid ODC activity reaching a maximum at 4 hr (Figure 6). This increased ODC activity has been shown to be blocked by actinomycin D or cycloheximide indicating de novo enzyme synthesis rather than activation of pre-existing enzyme (44). Ornithine decarboxylase activity as well as S-adenosyl-L-methionine decarboxylase (SAMD) activity increased in a biphasic pattern during the 14 days of PTU administration (Figure 7). Ornithine decarboxylase activity was elevated as early as 1 day reaching a maximum (7-fold increase) by 3 days followed by a marked decline to near control levels at 4 days. A second peak of ODC (3-fold increase) was seen at 6 days with a subsequent return to basal activity throughout the remainder of the treatment. S-adenosyl-L-methionine decarboxylase activity showed a biphasic pattern with maximum activities at 3 and 9 days, also returning to control levels at 4 and 14 days. S-adenosyl-L-methionine
Figure 6. Effect of thyroid stimulating hormone (TSH) administration (1 Unit/100 g BW given i.p.) on the time course of ODC activity in the thyroid of intact rats at various times after administration — Each point represents the mean ± S.E.M. of duplicate determinations in 5 separate rats.
Figure 7. Effect of propylthiouracil (PTU) on the activities of ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (SAMD) in the thyroid of the rat — Rats were given PTU at 0.02% in their drinking water for various time periods. Data are expressed as the mean ± S.E.M. of from 5-10 animals. Ornithine decarboxylase activity was significantly elevated above controls at days 1, 2, 3, 4, and 6 (p < 0.01) whereas SAMD activity was significantly different from controls only at days 3, 9, and 11 (p < 0.01).
decarboxylase was highest at day 9 with a 4-fold elevation of enzyme activity.

Cyclic AMP-Dependent Protein Kinases in Rat Thyroid Hypertrophy and Hyperplasia

The cyclic AMP-dependent protein kinase activity ratio (-cAMP/+CAMP) followed a very similar biphasic pattern during thyroid growth as that seen with ODC activity (Figure 8). The first peak in cyclic AMP-dependent protein kinase activity ratio occurred at 1 day and preceded the peak of ODC activity seen at 3 days. The dramatic decrease in cyclic AMP-dependent protein kinase activation seen at 3 days preceded the marked decline in ODC activity seen at 4 days. The highest activity ratio occurred at 6 days (150% of control) and was maintained at this level through 11 days falling to control again at 14 days.

In contrast to the biphasic pattern in cyclic AMP-dependent protein kinase activity ratio, the total supernatant cyclic AMP-dependent protein kinase activity (+cAMP) showed a different pattern. Total cyclic AMP-dependent protein kinase activity increased to 150% of control by 4 days and remained elevated until 14 days when it dropped to control levels (Figure 9).

Soluble rat thyroid cyclic AMP-dependent protein kinase was separated into two forms designated types I and II by DEAE-cellulose chromatography (Figure 10B). Type I and II elute from the column at 20-60 mM and 160-200 mM osmolarity, respectively, consistent with cyclic AMP-dependent protein kinases from other tissues. The predominant form of the enzyme in thyroid is type II comprising 90% of the total cyclic AMP-dependent protein kinase. Marked and selective alterations in the
Figure 8. Effect of administration of propylthiouracil (PTU) on the cyclic AMP-dependent protein kinase activity ratio (−cAMP/+cAMP) in the thyroid of the rat — Each point represents the mean ± S.E.M. of at least 5 rats. Asterisks indicate data that differ significantly from controls (p < 0.05).
Figure 9. Effect of propylthiouracil administration on the total supernatant activity of cyclic AMP-dependent protein kinase in the thyroid of the rat (measured in the presence of 10 μM cAMP) — Each point represents the mean ± S.E.M. of measurements of at least 5 animals. Asterisks indicate data significantly different from control (*p < 0.01; **p < 0.05).
Figure 10. Elution profile on DEAE-cellulose chromatography columns of cyclic AMP-dependent protein kinases from the rat adenohypophysis (A) and thyroid (B) — Measured in the presence, o–o, or absence, o–o, of 10 μM cyclic AMP. The protein kinase activity eluting first (fractions 5-7) is designated type I and represents 10% of the total protein kinase in both tissues. Type II elutes later (fractions 14-22) and comprises the remaining 90% of the protein kinase activity. The line indicates the 0-400 mM NaCl gradient which was initiated following 15 mls of washing buffer (see Methods). One ml fractions were collected.
amounts of type I and II protein kinase were seen during the 14-day growth period (Figure 11). Both type I and II remained at control levels for the first 2 days (a time when an increase in activation occurs) but increased conspicuously from 2 to 4 days reaching 150% and 175% of control, respectively. Type I remained elevated throughout the 14-day period whereas type II sharply declined after 4 days returning to control by 9 days. Type II again increased (150% of control) at 14 days.

Responsiveness of the Thyroid Following Hypophysectomy and Chronic Thyrotropin Stimulation in Rats

Thyroids from rats hypophysectomized for up to 2 weeks showed a significant decrease in total cyclic AMP-dependent protein kinase activity (Table 1). Thyroid supernatants from rats hypophysectomized for 1 week showed a total cyclic AMP-dependent protein kinase activity of 80% of control (data not shown) with a corresponding decrease in the amounts of both type I and II (61 and 77% of control respectively; p < 0.05). Absence of TSH for 2 weeks resulted in a further decrease in cyclic AMP-dependent protein kinase activity. Type I and type II cyclic AMP-dependent protein kinase activity decreased to 57 and 55% of control respectively (Table 1).

The well-known subsensitivity of the thyroid to exogenously administered TSH following removal of the trophic effect of endogenous TSH was tested in rats hypophysectomized for 2 weeks. There was a dramatic reduction in the ODC response to a single injection of TSH (3-fold compared to 20-fold in controls (Figure 12). Basal thyroid ODC activity was similar in both control and hypophysectomized rats. The
Figure 11. Effect of propylthiouracil administration on the total amounts of type I and II cyclic AMP-dependent protein kinase in the rat thyroid -- Data are presented as the mean ± S.E.M. of 5 columns (each column had applied to it a supernatant containing 3 thyroid glands). Each value was determined by adding the total enzyme activity under each peak following chromatography on DEAE as shown in Figure 10 and expressed as pmol of $^{32}$PO$_4$ incorporated into histone/min/mg protein added to the column. Asterisks represent data significantly different from control (*p < 0.01; **p < 0.05).
Figure 11. Effect of propylthiouracil administration on the total amounts of type I and II cyclic AMP-dependent protein kinase in the rat thyroid.
Table 1. Cyclic AMP-dependent protein kinase type I and type II activities in the rat thyroid following hypophysectomy.

<table>
<thead>
<tr>
<th>Experimental group (N)</th>
<th>Thyroid protein kinase activity pmol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>Control (3)</td>
<td>46.9 ± 0.96</td>
</tr>
<tr>
<td>1 week after hypophysectomy (3)</td>
<td>28.6 ± 1.41*</td>
</tr>
<tr>
<td>2 weeks after hypophysectomy (1)</td>
<td>25.7</td>
</tr>
</tbody>
</table>

*Data significantly different from control (p < 0.05). Each N composed of 7 pooled thyroids except at 2 weeks when 10 glands were pooled.
Figure 12. Effect of hypophysectomy (hypox) on the thyroid ODC response to exogenous TSH — Male Sprague Dawley rats (200-250 g) hypophysectomized for 2 weeks were administered TSH (1 Unit/100 g BW i.p.) and ODC measured 4 hrs after injection. Bars represent the mean ± S.E.M. of 5 determinations. Both the control + TSH groups and the hypox + TSH groups are significantly different from controls (p < 0.05).
thyroid gland, despite being deprived of its trophic maintenance via TSH for 2 weeks, showed no detectable decrease in wet weight (data not shown).

Chronically stimulated thyroids also have been shown to be subsensitive or refractory to exogenous TSH administration (15). However, thyroids stimulated for 14 days by high endogenous TSH (via PTU administration) responded in a normal manner when ODC induction was measured 4 hr following injection of TSH (Figure 13). The ODC activity in the 14-day treated glands had returned to control levels as also found in an earlier study (see Figure 4).

Chicken Thyroid Hypertrophy During Propylthiouracil (PTU) Treatment

The thyroid gland of White Leghorn hens was much less responsive to PTU than in rats. The thyroid was only 160% of control wet weight after 21 days exposure to the drug (Figure 14). After 7 days on PTU the thyroid was enlarged (135% of control) but histologically resembled control glands (Figure 15). However, by 21 days, marked cellular hypertrophy was evident with only a small decrease in follicular colloid. There was only slight histological evidence of hyperplasia present after 21 days (Figure 15). Thus, the chicken thyroid represents a much slower growth system than does the rat thyroid providing an opportunity to look more specifically at cellular hypertrophy as opposed to hyperplasia.

An increase in cyclic AMP-dependent protein kinase activity ratio was seen at 7 days (150% of control) followed by a return to control by 14 days in much the same manner as occurred in the early
Figure 13. Effect of chronic propylthiouracil stimulation on the ODC response of the thyroid to exogenous TSH in rats. Rats were administered 0.02% PTU in their drinking water for 14 days and given TSH (1 Unit/100 g BW i.p.). ODC activity was measured at 4 hr. Data are expressed as the mean ± S.E.M. of 5 determinations. There were no significant differences between control + TSH and 14 day PTU + TSH or between control and 14 day PTU groups.
Figure 14. Effect of propylthiouracil (PTU) on the thyroid wet weight in chickens -- White Leghorn hens were given PTU at 0.1% in their feed for various time periods. Each bird received approximately 100 mg PTU per day. Data are presented as mean ± S.E.M. of 5 birds for each point.
Figure 15. Histological changes in the chicken thyroid following propylthiouracil (PTU) treatment — Glands were stained by a standard hematoxylin and eosin procedure and magnified 400 X. (A) Control, (B) 7 days on PTU, (C) 21 days on PTU.
Figure 15.--Continued
hypertrophy phase (0-3 days) of the rat thyroid (Figure 16). The thyroid basal cyclic AMP-dependent protein kinase activity ratio was found to be $0.46 \pm 0.01$ in the chicken as compared to $0.31 \pm 0.02$ in rats possibly reflecting an important species characteristic. Similar to what was seen in the rat thyroid, the total supernatant protein kinase activity (+cAMP) increased during the 21 days of PTU treatment (180% of control; Figure 17). Tissue homogenized in buffer in the presence or absence of 150 mM KCl showed a similar pattern of increase in total cyclic AMP-dependent protein kinase activity (Figure 17). The inclusion of 150 mM salt has been shown to prevent nonspecific alterations in compartmentation of enzyme during homogenization (140). A slight inhibition of cyclic AMP-dependent protein kinase activity was seen due to the presence of salt (approximately 10-20% inhibition).

DEAE-cellulose chromatography of chicken thyroid 10,000 x g supernatant resulted in an identical pattern of type I and II cyclic AMP-dependent protein kinase activities (10% type I, 90% type II as seen in the rat thyroid; Figure 18B). Only type I protein kinase increased by 7 days of PTU treatment (120% of control, $p < 0.05$) with no change in the amount of type II cyclic AMP-dependent protein kinase (Figure 19). By 14 days, both types I and II were increased in amount of activity (300% and 200% of control, respectively) as occurred in the rat thyroid between 2 and 4 days. Type II activity remained elevated at 21 days whereas type I returned to control.
Figure 16. Effect of the administration of propylthiouracil on the thyroid cyclic AMP-dependent protein kinase activity ratio (-cAMP/+cAMP) in chickens -- Each point represents the mean ± S.E.M. of 5 determinations. Asterisks indicate data significantly different from controls (p < 0.05).
Figure 17. Effect of propylthiouracil administration on the total supernatant activity of cyclic AMP-dependent protein kinase in chickens — Measurements were made in the presence of 10 μM cAMP in buffer containing either no KCl (-----) or 150 mM KCl (o--o). Differences between measurements made in 0 or 150 mM KCl buffers were significant only at 14 days (p < 0.05).
Figure 18. Elution profile on DEAE-cellulose chromatography columns of cyclic AMP-dependent protein kinase from the chicken adenohypophysis (A) and thyroid (B). Measurements were made in the presence (---) or absence (----) of 10 µM cAMP. The profiles of type I and type II protein kinase are very similar to that seen in the corresponding rat tissues (see Figure 10).
Figure 19. Effect of propylthiouracil administration on the total amounts of type I and II cyclic AMP-dependent protein kinase in the chicken thyroid — Data are presented as the mean ± S.E.M. of 3 columns (each column run with pooled glands from 3 chickens). The procedure is the same as described in Figure 11 and in the Materials and Methods. Asterisks represent data significantly different from controls (*p < 0.01, **p < 0.05).
Figure 19. Effect of propylthiouracil administration on the total amounts of type I and II cyclic AMP-dependent protein kinase in the chicken thyroid.
Trophic Effects of Thyrotropin-Releasing Hormone (TRH) and Lowered Plasma Levels of Thyroid Hormone on the Adenohypophysis of the Rat and Chicken

Cyclic AMP-Dependent Protein Kinases in the Adenohypophysis During Propylthiouracil Administration

The adenohypophysial cyclic AMP-dependent protein kinase activity ratio increased to 0.40 ± 0.030 from a basal level of 0.27 ± 0.006 (p < 0.01) after 1 day of PTU treatment (Figure 20). Protein kinase remained activated through 4 days but declined to control by 6 days. The activity ratio again increased on day 11 and 14. The pattern is biphasic, similar to what was found in the rat thyroid.

DEAE-cellulose chromatography of adenohypophysial 10,000 x g supernatant in both rats and chickens resulted in type I and type II forms of cyclic AMP-dependent protein kinase in a ratio similar to that found in the thyroid gland, i.e., 90% type II, 10% type I (Figures 10A and 18A). No changes were observed in the amounts of rat adenohypophysial type I or type II after 2 days of PTU stimulation. However, by 14 days type II had increased to 160% of control with no alteration in type I (Table 2). In contrast, type II in chicken adenohypophysis was only 68% of control with again no alteration in type I cyclic AMP-dependent protein kinase at 14 or 21 days of PTU treatment (Table 3).

Effects of Thyroid State on Ornithine Decarboxylase Activity of the Adenohypophysis of the Rat and Chicken

Ornithine decarboxylase activity in the rostral lobe of the adenohypophysis of the chicken was elevated significantly 3 to 4 hr after the injection of 0.5 ng/kg of TRH (p < 0.05; Figure 21). Maximal activity was detected at 4.5 hr and declined to the control value and
Figure 20. Effect of propylthiouracil administration on the cyclic AMP-dependent protein kinase activity ratio (-cAMP/+cAMP) of the rat adenohypophysis — Each point represents the mean ± S.E.M. of at least 5 rats. Asterisks indicate data significantly different from controls (*p < 0.01, **p < 0.05).
Table 2. Cyclic AMP-dependent protein kinase type I and type II activities in the rat adenohypophysis following propylthiouracil (PTU) treatment.

<table>
<thead>
<tr>
<th>Experimental group (N)</th>
<th>Adenohypophysial protein kinase activity pmol/min/mg protein</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
<td>Type II</td>
</tr>
<tr>
<td>Control (3)</td>
<td>212.7 ± 35.1</td>
<td>1246.7 ± 110.3</td>
</tr>
<tr>
<td>2 day PTU (3)</td>
<td>175.6 ± 27.9</td>
<td>1357.6 ± 18.8</td>
</tr>
<tr>
<td>14 day PTU (3)</td>
<td>241.5 ± 26.9</td>
<td>1975.4 ± 62.1*</td>
</tr>
</tbody>
</table>

*Data significantly different from control (p < 0.01). Each N represents the pooled glands from 5 rats.

Table 3. Cyclic AMP-dependent protein kinase type I and type II activities in the chicken adenohypophysis following propylthiouracil (PTU) treatment.

<table>
<thead>
<tr>
<th>Experimental group (N)</th>
<th>Adenohypophysial protein kinase activity pmols/min/mg protein</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
<td>Type II</td>
</tr>
<tr>
<td>Control (3)</td>
<td>240.5 ± 32.5</td>
<td>2035.4 ± 123.4</td>
</tr>
<tr>
<td>14 day PTU (3)</td>
<td>161.0 ± 28.0</td>
<td>1380.3 ± 17.6*</td>
</tr>
<tr>
<td>21 day PTU (3)</td>
<td>203.2 ± 28.2</td>
<td>1405.2 ± 15.7*</td>
</tr>
</tbody>
</table>

*Data significantly different from control (p < 0.01). Each N represents the pooled glands from 5 chickens.
Figure 21. Ornithine decarboxylase activity (ODC) in the chicken adenohypophysis at various times after thyrotropin-releasing hormone (TRH) administration (0.5 ng/kg i.p.) — Each point represents the mean ± S.E.M. of 4 determinations except at 4.5 hr where 7 determinations were made. Rostral lobe (---o) and caudal lobe (o---o) of chicken adenohypophysis. Enzyme activity was significantly elevated above control levels at 3.5 and 4.5 hr (p < 0.05). Control levels of ODC activity for both rostral and caudal lobes varied from 20 to 90 pmol/hr/mg protein.
below within 5.5 to 7 hr. In the caudal lobe, ODC activity was not
significantly altered at any of the time intervals. Regression
analysis of the induction of ODC by TRH showed a significant linear
dose response (p < 0.01) from 1 to 10 ng in the rostral lobe (Figure
22). However, ODC activity in the caudal lobe was not significantly
different from controls at any dose of TRH administered. Thyrotropin-
releasing hormone (TRH) doses greater than 10 ng were not administered
due to extrathyrotropic effects known to occur with higher doses (141).

In contrast to the chicken, no induction of ODC by TRH was
observed in rats from 0 to 6 hr after injection. The dose of TRH
used (250 ng/100 g BW) was shown to be effective in causing TSH
release 15 min after injection (Table 4). There was even a depression
of ODC at 2 hr.

Following surgical thyroidectomy, plasma levels of $T_3$ and $T_4$
declined rapidly (Figure 23A). Consistent with the accepted view of TSH
stimulation via removal of negative feedback at the pituitary, plasma
TSH levels showed a corresponding pattern of increase. Thyrotropin was
elevated 4-fold within 1 day and reached levels 20-fold above controls
within 4 days (Figure 23B). Ornithine decarboxylase activity in the
adenohypophysis increased with increases in TSH plasma levels and was
consistently 1.5- to 2-fold greater than sham-operated controls with
the single exception of 3 days, at which time the activity was
decreased (Figure 23C).

In the chicken, feeding the antithyroid drug methimazole,
produced a similar 2-fold increase in ODC in both the rostral and
caudal lobes (p < 0.025) of the adenohypophysis (Figure 24, Expt. 3).
Figure 22. Effect of different amounts of thyrotropin-releasing hormone (TRH) on ornithine decarboxylase at 4 hr in the rostral (R) and caudal (C) lobes of the adenohypophysis of the chicken — Each bar represents the mean ± S.E.M. of 3 determinations.
Table 4. Plasma hormone levels and ornithine decarboxylase activity in the adenohypophysis of the rat following TRH administration.

<table>
<thead>
<tr>
<th>Hours after TRH (µg/100 ml)</th>
<th>TSH (µg/100 ml)</th>
<th>T₃ (µg/100 ml)</th>
<th>T₄ (µg/100 ml)</th>
<th>Ornithine decarboxylase (pmol of ¹⁴CO₂/mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43.69 ± 12.37ᵃ</td>
<td>107.21 ± 9.66</td>
<td>5.34 ± 0.44</td>
<td>48 ± 7.6</td>
</tr>
<tr>
<td>0.25</td>
<td>115.56 ± 24.35*</td>
<td>118.36 ± 8.38</td>
<td>6.16 ± 0.27</td>
<td>35 ± 10.7</td>
</tr>
<tr>
<td>2</td>
<td>25.13 ± 3.97</td>
<td>123.70 ± 9.65</td>
<td>5.08 ± 0.82</td>
<td>15 ± 3.2**</td>
</tr>
<tr>
<td>4</td>
<td>28.93 ± 5.67</td>
<td>96.69 ± 4.09</td>
<td>5.38 ± 0.46</td>
<td>28 ± 6.8</td>
</tr>
<tr>
<td>4 (saline)</td>
<td>71.07 ± 10.21**</td>
<td>92.47 ± 6.53</td>
<td>4.89 ± 0.61</td>
<td>34 ± 6.9</td>
</tr>
<tr>
<td>6</td>
<td>36.21 ± 7.86</td>
<td>90.77 ± 8.64</td>
<td>4.99 ± 0.53</td>
<td>28 ± 5.2</td>
</tr>
</tbody>
</table>

ᵃEach value represents the mean ± S.E.M. of at least 5 rats.

*Data differ from control (p < 0.05).

**Data differ from control (p < 0.01).
Figure 23. Triiodothyronine (T₃), thyroxine (T₄), and thyroid-stimulating hormone (TSH) after thyroidectomy and corresponding ornithine decarboxylase (ODC) activity in the adenohypophysis of the rat — (A) o○○, T₃; o—o, T₃ sham-operated controls; Δ—Δ, thyroxine; Δ—Δ, thyroxine controls. (B) o—o, TSH; o—o, TSH controls. (C) Ornithine decarboxylase. The initial activity of ODC was 13.6 ± 4.06 pmol/hr/mg protein. ODC activity was significantly elevated at 1, 2, 4, and 7 hr (p < 0.005, p < 0.05, p < 0.001, and p < 0.025, respectively).
Figure 23. Triiodothyronine ($T_3$), thyroxine ($T_4$), and thyroid-stimulating hormone (TSH) after thyroidectomy and corresponding ornithine decarboxylase (ODC) activity in the adenohypophysis of the rat.
Figure 24. Ornithine decarboxylase activity in the rostral (R) and caudal (C) lobes of the adenohypophysis of the chicken at 4 hr after thyroxine (T₄) administration and after methimazole treatment. Each bar represents the mean ± S.E.M. of 3 determinations (6 chickens) in experiments 1 and 2, and 6 determinations (12 chickens) in experiment 3. All T₄ data were significantly different from controls in both lobes, rostral (p < 0.05) and caudal (p < 0.005). Methimazole (0.1%) in feed was given for 15 days. Methimazole data from rostral and caudal lobes were significantly different from controls (p < 0.025).
Thyroxine administration (10 µg/chicken) significantly increased ODC activity 2- to 4-fold in the rostral lobe and 2- to 8-fold in the caudal lobe (Figure 24, Expts. 1 and 2). The control level of ODC in the adenohypophysis of the chicken varied between experiments, probably reflecting individual and environmental conditions.

**Effects of Triiodothyronine on the Polyamine Biosynthetic Enzymes in Liver and Kidney**

Effect of Long-Term Propylthiouracil Treatment on Growth of Various Tissues

Chronic administration of 0.02% PTU to weanling rats (22 days of age) for 6 weeks resulted in a characteristic reduction in body growth rate (Figure 25A). After 6 weeks of PTU treatment, rats were only 70% of control body weight. The hypothyroid state induced by PTU resulted in not only general depression of body growth but had specific effects on various organs. There was a selective decrease in both liver and kidney organ weight/body weight ratios with wet weights being 80% and 95% of control, respectively (Figure 25B). This is consistent with the fact that both the liver and kidney are highly responsive to thyroid hormones and possess the greatest number of T₃ nuclear receptors (127). The ventral prostate with little or no response to thyroid hormones showed no decrease in organ to body weight ratio as a result of the chronic deprivation of T₃ and T₄. The thyroid gland was greatly enlarged (400% of control) due to the chronically high TSH levels indicating the effectiveness of the PTU treatment in lowering T₃ and T₄ levels in this study.
Effect of long-term propylthiouracil (PTU) treatment on body weight and growth of various tissues in the rat — Weanling Sprague-Dawley rats (male, 22 days old) were administered 0.02% PTU in their drinking water for 6 weeks and body weights determined at various intervals. After 6 weeks the animals were sacrificed and the wet weights of several organs determined. Each point represents the mean ± S.E.M. of from 6-12 determinations. (A) Weight gain of control rats (●—●) compared to PTU treated rats (○—○). (B) Open bars (control), cross-hatched bars (PTU treated). All treatment groups are significantly different from control (p < 0.01) except for the prostate.
Figure 25. Effect of long-term propylthiouracil (PTU) treatment on body weight and growth of various tissues in the rat.
Basal activities of ODC and SAMD were significantly decreased in both liver and kidney following 6 weeks of PTU treatment (Table 5). The most dramatic effect was on kidney ODC where enzyme activity was only 18% of control.

Effects of Triiodothyronine and/or Aminophylline on the Activation of Cyclic AMP-Dependent Protein Kinases and the Induction of Ornithine Decarboxylase in Rat Liver

Triiodothyronine (\(T_3\), 300 \(\mu\)g/kg) administered to intact rats resulted in a 6-fold induction of ODC in the liver within 4 hr (Figure 26). Ornithine decarboxylase was elevated significantly \((p < 0.001)\) as early as 3 hr after administration and returns to near control levels within 6 hr. The administration of 100 \(\mu\)g/kg of \(T_3\) was the minimum dose sufficient to significantly elevate ODC by 4 hr, and the maximum induction of ODC was detected after the administration of 300 \(\mu\)g/kg (Figure 27). There was no detectable increase in the activity ratio of cyclic AMP-dependent protein kinase measured from 0 to 4 hr after the injection of \(T_3\) (Figure 26).

The administration of aminophylline (200 \(\mu\)mol/kg) resulted in a 10-fold induction of ODC in the liver within 4 hr with an identical time course to that detected after \(T_3\) administration. Cyclic AMP-dependent protein kinase activation as evidenced by an increase in activity ratio occurred as early as 20 min after injection and remained elevated for the first 2 hr, prior to any increase in ODC activity (Figure 28). When \(T_3\) (300 \(\mu\)g/kg) was administered simultaneously with aminophylline (200 \(\mu\)mol/kg), as described above, there was a 16-fold elevation of ODC activity within 4 hr (Figure 29).
Table 5. Basal ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities in the liver and kidney of rats following a 6-week treatment with propylthiouracil (PTU).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ornithine decarboxylase pmols $^{14}$CO$_2$/30 min/mg protein</th>
<th>S-adenosyl-L-methionine decarboxylase pmols $^{14}$CO$_2$/30 min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PTU treated</td>
</tr>
<tr>
<td>Liver</td>
<td>7.7 ± 1.7</td>
<td>2.2 ± 0.7*</td>
</tr>
<tr>
<td>Kidney</td>
<td>182.4 ± 35.1</td>
<td>32.0 ± 7.3**</td>
</tr>
</tbody>
</table>

Each control value represents the mean ± S.E.M. of 6 rats and PTU treated groups represents the mean ± S.E.M. of 12 rats.

*Data differ from control (p < 0.05).

**Data differ from control (p < 0.025).
Figure 26. Temporal changes in the activation of cyclic AMP–dependent protein kinase and the activity of ornithine decarboxylase (ODC) in rat liver following T₃ administration (300 μg/kg, i.p.) — The single 1-hr point represents the degree of protein kinase activation in rat liver after administration of 3-isobutyl-1-methylxanthine (MIX). The data represent the mean ± S.E.M. of 5 to 10 duplicate determinations on separate rats.
Figure 27. Ornithine decarboxylase activity in rat liver 4 hr after the administration of various amounts of T₃ -- Data presented as the mean ± S.E.M. of 5-10 duplicate determinations on different rats.
Figure 28. Time course of activation of cyclic AMP-dependent protein kinase and the activity of ornithine decarboxylase in rat liver following aminophylline administration (200 μmol/kg, i.p.) — The data represent the mean ± S.E.M. of 5 to 10 duplicate determinations on different rats.
Figure 29. Ornithine decarboxylase activity 4 hr after the injection of triiodothyronine (T₃, 300 μg/kg, i.p.), aminophylline (200 μmol/kg, i.p.), or T₃ plus aminophylline in the above doses — Each bar represents the mean ± S.E.M. of duplicate determinations in 5 animals.
Effects of Somatostatin on the Ability of Triiodothyronine to Induce Ornithine Decarboxylase in Rat Liver

Previous studies had indicated a similar effect of growth hormone on ODC activity in liver as reported here for T₃ and aminophylline (142, 143). Triiodothyronine has a well known effect on the pituitary content of growth hormone and thus it became important to eliminate the possibility that T₃ action on liver ODC was indirectly mediated by growth hormone.

Pretreatment of rats with somatostatin in a protamine-zinc mixture known to lower plasma growth hormone levels by blocking its pituitary release had no effect on the ability of T₃ to induce ODC within 4 hr (Figure 30). In fact, somatostatin-pretreated animals showed a greater induction of ODC in response to T₃ administration.

Effects of Inhibitors of RNA and Protein Synthesis on the Activity of Ornithine Decarboxylase after Triiodothyronine or Aminophylline

Either cycloheximide or actinomycin D administered simultaneously with T₃ totally inhibited the increase in ODC activity observed at 4 hr (Table 6). The induction of ODC after administration of various phosphodiesterase inhibitors has previously been shown to be blocked by either cycloheximide or actinomycin D (46).

Triiodothyronine Induction of Kidney Ornithine Decarboxylase

Triiodothyronine (300 μg/kg, i.p.) resulted in a 3-fold induction of ODC in the kidney in a similar time course as that seen in the liver (Figure 31). However, the maximum ODC activity occurred at 6 hr as compared to 4 hr in the liver. A dose-response relationship
Figure 30. The effect of somatostatin pretreatment on the induction of hepatic ornithine decarboxylase 4 hr after T₃ administration (300 µg/kg, i.p.) — Somatostatin was prepared as described in Methods and administered 3 hr prior to T₃ injection. Each bar represents the mean ± S.E.M. of duplicate determinations of 5 animals.
Table 6. Effect of inhibitors of RNA and protein synthesis on the triiodothyronine stimulation of liver ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Liver enzyme activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ornithine</td>
<td>S-adenosyl-L-methionine decarboxylase</td>
</tr>
<tr>
<td></td>
<td>decarboxylase</td>
<td>(18 hr)</td>
</tr>
<tr>
<td></td>
<td>(4 hr)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.7 ± 1.6</td>
<td>22.4 ± 2.9</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>61.6 ± 15.8*</td>
<td>39.3 ± 6.3*</td>
</tr>
<tr>
<td>Triiodothyronine + actinomycin D</td>
<td>14.1 ± 2.0</td>
<td>4.9 ± 2.1*</td>
</tr>
<tr>
<td>Triiodothyronine + cycloheximide</td>
<td>12.8 ± 2.2</td>
<td>--</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M. of 4 determinations.

*Data significantly different from control (p < 0.05).
Figure 31. Temporal changes in the activity of ornithine decarboxylase in rat kidney following triiodothyronine administration (300 μg/kg, i.p.) — The data represent the mean ± S.E.M. of at least 5 determinations.
was demonstrated for kidney ODC induction by T₃ with 100 μg/kg, i.p., the minimum effective dose and 300 μg/kg, i.p., the most effective dose (Figure 32). This was identical to the T₃ dose-response found in the liver.

**Triiodothyronine Induction of S-Adenosyl-L-Methionine Decarboxylase (SAMD) Activity in Rat Liver**

Triiodothyronine (300 μg/kg, i.p.) resulted in a 2-fold elevation in liver SAMD by 12 hr (Figure 33). The SAMD activity was significantly increased (150%, p < 0.05) as early as 5 hr following the injection. The increased SAMD activity seen at 18 hr was completely blocked by the simultaneous injection of actinomycin D (Table 6). Actinomycin D not only blocked the SAMD increase at 18 hr but lowered the activity of this enzyme to 20% of the control basal level.
Figure 32. Kidney ornithine decarboxylase activity 4 hr after administration of various amounts of triiodothyronine. Data presented as mean ± S.E.M. of 5 determinations. All treatment values are significantly different from control (p < 0.01).
Figure 33. Temporal changes in the activity of S-adenosyl-L-methionine decarboxylase activity in rat liver following triiodothyronine administration (300 μg/kg, i.p.)—The data represent the mean ± S.E.M. of 5 determinations.
DISCUSSION

The induction of ornithine decarboxylase (ODC) by thyrotropin (TSH) in the rat thyroid is similar to that found in other hormone-stimulated tissues, i.e., growth hormone stimulation of rat liver (142, 143), testosterone stimulation of the ventral prostate (132), luteinizing hormone on the ovary (144, 145), 17β-estradiol stimulation of the uterus and oviduct (146, 147), β-ecdysone effect on Cecropia larvae (148), thyrotropin-releasing hormone effects on the adenohypophysis of the chicken (149). Administration of TSH resulted in a 20-fold induction of ODC within 4 hr. This is similar to the report by Zusman and Burrow (44) who found a 22-fold induction of ODC within 6 hr with a similar dose of TSH administered intramuscularly. The 2-hr delay in their study could be due to a slower absorption of TSH after intramuscular administration compared to intraperitoneal administration. Yu et al. (150) reported a 20-30-fold increase in ODC activity of the mouse thyroid at 4 hr following 10 mU of TSH, i.p. A similar time course, with maximal ODC activity occurring at 4 hr (3- to 5-fold), was demonstrated in vitro following addition of TSH or dibutyryl-cyclic AMP to the incubated thyroid (43). The effects of both TSH and dibutyryl-cyclic AMP to increase ODC activity in the thyroid have been shown to be completely blocked by actinomycin D or cycloheximide, both in vivo and in vitro (43, 44). In addition, the stimulation of ODC by TSH or 3-isobutyl-1-methylxanthine has been shown to involve an increase in
ornithine deccarboxylase synthesis as evidenced by an increase in the immunoprecipitable enzyme protein (151).

Propylthiouracil (PTU) is a member of a family of compounds known as thiourelenes. These compounds contain a thioamide group, S=C=N=, and specifically inhibit the incorporation of iodide into the precursors of T\textsubscript{3} and T\textsubscript{4}. The biochemical mechanism of this PTU effect has been shown to involve a competitive inhibition of a thyroidal peroxidase enzyme responsible for the synthesis of T\textsubscript{3} and T\textsubscript{4} (152). In addition, PTU is capable of blocking the deiodonation of T\textsubscript{4} to T\textsubscript{3} thereby very rapidly decreasing plasma T\textsubscript{3} levels (153).

Addition of PTU to the drinking water of rats has been shown to result in a fluctuating pattern of plasma as well as pituitary content of TSH (154). This pattern of plasma TSH fluctuation was strikingly similar to the fluctuation in ODC activity in the thyroid reported in this study, i.e., both ODC and plasma TSH increase at 1 day and reach a peak level at 3 days followed by a rapid decline returning to control by 14 days.

The pattern of ODC activity reported in this study with maximum activity at 3 days (7-fold) compares to that reported by other investigators following administration of methylthiouracil in the drinking water of rats. Richman et al. (155) reported a maximum ODC activity at 2 days (15-fold) whereas Matsuzaki and Suzuki (156) reported a maximum ODC activity occurring at 4 days (8- to 18-fold) following initiation of goitrogen treatment. The increased SAMD activity at 3 days (150% of control) reported in this study is similar to the maximal activity found by Matsuzaki and Suzuki (200% of control).
by 4 days. An additional peak of SAMD activity (4-fold) occurred at 9 days and returned gradually to control levels by 14 days. Matsuzaki and Suzuki reported no further increases in SAMD following the initial 4-day peak, but failed to make measurements between 7 and 15 days likely missing this second peak in SAMD activity. Thus, the demonstration that TSH can induce ODC together with the similarity in the plasma TSH level and thyroid ODC activity described during goitrogen treatment substantiates the role of TSH in the regulation of ODC in the thyroid.

A recently discovered inhibitory protein of ODC could also be a contributing factor in the fluctuations in ODC activity seen during PTU treatment. This nondialyzable heat-labile protein inhibitor of ODC first described by Fong, Heller, and Canellakis (157) has been demonstrated in thyroid tissue by Friedman et al. (158). They found a protein inhibitor of ODC in the thyroid that was produced in vivo by multiple injections of the polyamines, putrescine or spermidine, into rats and in vitro by incubating bovine thyroid slices with polyamines. Multiple polyamine injections followed by the production of the ODC inhibitor was shown to inhibit the increase in ODC activity and thyroid weight produced by goitrogens (159). Therefore, the TSH-promoted stimulation of polyamine accumulation could result in the production of a ODC inhibitor which could feedback and decrease further polyamine synthesis via a direct control of ODC, the rate-limiting enzyme in the pathway.

Increased activities of ODC and SAMD have been shown to lead to an accumulation of polyamines in the thyroid (159). Following goitrogen treatment, RNA and spermidine accumulate in parallel as is common in
many growth systems (159). Thyrotropin and dibutyryl-cyclic AMP have been demonstrated to result in an increase in RNA polymerase I activity, the enzyme responsible for rRNA synthesis (122). It seems likely that the ODC induced by TSH or dibutyryl-cyclic AMP acts both to regulate polyamine biosynthesis and to regulate RNA polymerase I activity via serving as an initiation factor as has been postulated (94). The resulting increased polyamines may function to facilitate the production and transport of rRNA or in the formation of ribosomes thereby enhancing the protein synthesizing capability of the tissue.

The cyclic AMP-dependent protein kinase activity ratio in the thyroid followed a biphasic pattern similar to that seen with ODC in PTU-treated rats. A tight temporal correlation between cyclic AMP-dependent protein kinase activation and ODC induction has been reported in rat liver following partial hepatectomy (48). Both enzyme activities fluctuated in a biphasic pattern during the first 16 hr of liver regeneration with an increased protein kinase activity ratio preceding the increased ODC activity by approximately 2 hr. The temporal correlation as well as dose dependency relationship between the activation of cyclic AMP-dependent protein kinase and the induction of ODC has been extensively documented (39). Hormones or agents known to activate protein kinase also induce ODC within 1 to 3 hr following the peak of protein kinase activation. The mechanism for the protein kinase mediated induction of ODC is not known but could involve a hormone-stimulated cytoplasmic to nuclear translocation of either regulatory and/or catalytic subunits of protein kinase followed by a subsequent specific phosphorylation of chromatin-associated proteins.
related to transcriptional control of the ODC gene. This translocation has been demonstrated in luteinizing hormone (LH) stimulation of the ovary and cholinergic stimulation of the adrenal medulla and has been implicated as a major regulatory pathway of hormones on transcriptional processes (58, 59).

The thyroid of the chicken was found to be much less responsive to PTU administration than that of rats. Thyroids of chickens exposed to PTU for 21 days resembled histologically the glands of rats exposed for only 2 days. This could relate to the mode of drug administration, i.e., in the diet rather than in drinking water as in rats, or may reflect a species difference in drug sensitivity. The activation of protein kinase that occurred at 7 days in the thyroid of the chicken was at a time of a small amount of glandular hypertrophy (135% of control). This was similar to the early activation of protein kinase seen in the hypertrophy phase (0-3 days) in PTU-treated rats. Therefore, the effects of the early activation of protein kinase seen in both rats and chickens is likely involved with events specific to cellular hypertrophy and associated with protein and RNA synthesis. A gradual increase in protein kinase activity ratio has been reported in synchronized Chinese hamster ovary (CHO) cells as they progress through G₁ phase of the cell cycle (160). During G₁ phase there was a progressive increase in cellular RNA and protein with cellular hypertrophy which preceded the initiation of DNA synthesis that occurred in S phase. Acute exposure of bovine thyroid slices to TSH in vitro has been shown to result in a marked activation of protein
kinase in less than 5 min which is correlated temporally and in a dose-response manner to an elevation of cyclic AMP (117).

Identification of the functional consequences of the activation of cyclic AMP-dependent protein kinase related to the acute and long-term growth response requires assessment of the endogenous substrates for protein kinase. It is well known that TSH as well as dibutyryl-cyclic AMP can enhance labeled precursor incorporation into RNA and protein (120, 121). An enhanced ability of ribosomes to synthesize protein has been reported in thyroid tissue stimulated with TSH (161, 162).

Ribosomal proteins have been shown to be substrates for cyclic AMP-dependent protein kinase and could explain some of the translational effects of cyclic AMP on protein synthesis (33-36). Of particular relevance to the growth-promoting effects of TSH on the thyroid is the finding that TSH or cyclic AMP markedly enhance the phosphorylation of nuclear histone $F_1$ (163). Phosphorylation of histone $F_1$ has been reported after glucagen treatment in rat liver (56), during slime mold cell division (164), during cell cycle of hepatoma (HTC) cells (165), and in regenerating rat liver (166). The phosphorylation of histone $F_1$ has been correlated with cell growth and has been postulated to bring about structural changes in the chromatin necessary for transcription (167).

The thyroid of both chickens and rats were found to have identical patterns of type I and II protein kinase following DEAE-cellulose chromatography (90% type II and 10% type I). Our finding of increased amounts of both type I and II protein kinase during thyroid
hypertrophy and hyperplasia is supported by similar reports in other growth systems. Type I protein kinase is specifically increased during isoproterenol-induced cardiac hypertrophy (26). Types I and II protein kinase vary markedly during cell cycle. In synchronized CHO cells, type II increases in late G₁ followed by a dramatic decline as cells enter S phase. Type I protein kinase increases gradually throughout the cell cycle and declines following mitosis (15).

In the thyroid of the chicken, only type I protein kinase was increased at 7 days, a time of minimal gland hypertrophy with no evidence of hyperplasia. The 2-fold increase in both type I and II protein kinase seen at 14 days in chickens could relate to the thyroid epithelial cells traversing G₁ of the cell cycle prior to entry into S phase. The dramatic increase in types I and II observed in the thyroid of the chicken at 14 days was also observed in the thyroid of the rat at 4 days. No increase of type I kinase prior to a type II increase was detected in rat thyroid as was seen in the chicken. However, type I could have increased at 3 days since no enzyme measurements were made. In CHO cells only type I protein kinase has been reported to be present in early to mid-G₁ phase of the cell cycle, a time when there is a dramatic progressive increase in ODC activity. It has therefore been postulated that the early G₁-related induction of ODC is a result of the specific activation of type I cyclic AMP-dependent protein kinase (168). This idea is further indicated with the demonstration that along with increased ODC activity there is a selective activation of type I protein kinase following concanavalin A-stimulated lymphocyte mitogenesis (20).
The dramatic rise and fall in type II protein kinase in the rat thyroid coincides with the initiation of DNA synthesis. This same rise and fall of type II protein kinase also was seen during CHO cell cycle at the G↓1/S border (15). The accumulation of type II protein kinase in late G↓1 phase of the CHO cell cycle was inhibited by puromycin but not actinomycin D indicating that this increase was likely under translational control (15). The increase in type II seen in the rat thyroid from 9 to 14 days could represent the start of another sequence of cell cycle related events.

The finding of reduced amounts of type I and II protein kinases in thyroids from hypophysectomized rats indicates that one of the trophic effects of TSH could be maintenance of the proper intracellular concentration of cyclic AMP-dependent protein kinase. One consequence of the reduced levels of protein kinase could be a compromised response to agents working through the adenylate cyclase-cyclic AMP-dependent protein kinase axis. The demonstration of a diminished induction of ODC in the thyroid in response to exogenous TSH in hypophysectomized rats agrees with an earlier report in which TSH administration resulted in only a 5-fold induction of ODC in hypophysectomized rats compared to a 20-fold induction in intact animals (44). This altered response could also involve a decrease in TSH receptors.

That the return of ODC activity to control levels at 14 days of PTU treatment was due to effects other than a decreased sensitivity of the thyroid to TSH was demonstrated. Rat thyroid chronically stimulated for 14 days responded normally to exogenous TSH administration. Ornithine decarboxylase activity was measured 4 hr after TSH
injection. Thyroid glands from rats treated with PTU for up to 6 months failed to show the usual increase in cyclic AMP following addition of TSH in vitro (169). Apparently 14 days of stimulation was not long enough to bring about a desensitization of the thyroid to TSH. Therefore, the return of ODC to control levels at 14 days is likely due to a decrease in plasma TSH rather than a change in thyroid sensitivity to TSH.

The induction of ODC by TRH in the rostral lobe of the chicken adenohypophysis is similar temporally to that found in the rat thyroid after TSH treatment. Administration of as little as 0.5 ng/kg of TRH significantly increased ODC (p < 0.05) in the rostral lobe with no effect on the caudal lobe of the adenohypophysis. This finding supports the work of Radke and Chiasson (106) who have found a qualitative difference in the responsiveness of rostral and caudal thyrotrophs to TRH in vitro (106). Only rostral lobe portions incubated with TRH have increased levels of TSH. The failure of TRH to induce ODC in the rat adenohypophysis suggests a different sensitivity of the rat pituitary to TRH.

High plasma levels of TSH are associated with a hypothyroid state. May, Burrow, and Spaulding (170) have shown that plasma TSH increased nearly 4-fold after 5 days of propylthiouracil treatment in rats. During this same period, ODC activity of the pituitary increased 6-fold by 3 days with a subsequent decline toward control values by day 5. We obtained similar results after surgical thyroidectomy; TSH levels were 10-fold higher by 4 days and ODC was 2-fold of control within 1 day. ODC activity returned to control levels at 3 days when
TSH levels were shown to plateau. Thyrotropin (TSH) increased again from 3 to 4 days as did ODC activity. This close relationship between TSH plasma levels and pituitary ODC supports the association of ODC activity with subsequent TSH synthesis and release.

Methimazole-induced thyroidectomy in chickens produced a 2-fold elevation of ODC in both lobes of the adenohypophysis. Methimazole lowers plasma $T_3$ and $T_4$ levels by preventing their synthesis via the inhibition of iodination of tyrosine in the thyroid. The finding that the caudal lobe responds to lowered thyroid hormone levels but not to TRH suggests a dual control mechanism for TSH synthesis and release.

It has been shown that $[^3]$H TRH is bound specifically to receptors on the cell membrane of the thyrotroph (171). The biological effects of TRH have been considered by many to be mediated by cyclic AMP. In support for this concept, several investigators have reported increased intracellular cyclic AMP in the pituitary in response to TRH (107, 108, 172, 173). Following thyroidectomy in the rat, there was an elevation in cyclic AMP and an increased uptake and incorporation of $^{14}$C-labeled amino acids into TSH containing granules (107, 174) in the adenohypophysis.

The finding of an increased cyclic AMP-dependent protein kinase activity ratio in the rat adenohypophysis during propylthiouracil treatment is consistent with previous reports of alterations in cyclic AMP levels. In addition the pituitary has been shown to be more responsive to TRH in a hypothyroid animal (175). Griessen and Lemarchand-Béraud (154) have reported a dramatic 50% decrease in pituitary TSH content after one day of PTU administration in rats. The
TSH pituitary content gradually increased during the next 2 days but dropped again at 4 days only to increase again by 7 days. The early depletion of pituitary TSH was due to the extended release of hormone and the subsequent increases due to recovery biosynthesis of TSH. The early activation of protein kinase seen in this study (at 1 day) could be related to both the secretion process as well as initiating events leading to enhanced biosynthesis of TSH. Cyclic AMP administration has been shown to lead to a 40 to 100% increased incorporation of $^{32}$P phosphate into nine of 36 plasma membrane proteins in the bovine anterior pituitary (176). It was postulated by these investigators that the phosphorylation of these membrane components could lead to altered rates of membrane fusion and exocytosis thusly enhancing the secretion of TSH.

The early activation of cyclic AMP-dependent protein kinase in the rat adenohypophysis during PTU treatment also corresponds to the early increase in ODC activity seen following surgical thyroidectomy. This provides another instance of a correlation between protein kinase activation and ODC activity supporting the concept that ODC induction is a consequence of the activation of protein kinase. In addition to an accelerated synthesis of TSH in hypothyroidism, marked cellular changes occur in the pituitary thyrotrophs. The thyrotrophs were enlarged and exhibited an extensive endoplasmic reticulum (103). There is additional evidence that thyrotrhop hyperplasia occurs indicated by a 34 and 300% increase in pituitary DNA content and number of thyrotrophs following thyroidectomy in the rat respectively (104).
Both the chicken and rat adenohypophysis contain a similar relative amount of type I and II protein kinase (90% type II and 10% type I). This was identical to the relative amounts of type I and II found in the thyroid of these two species. The adenohypophysis had a 4- to 6-fold higher amount of cyclic AMP-dependent protein kinase compared to the thyroid, possibly related to its higher secretory activity. Alterations during PTU treatment in the adenohypophyses of both chickens and rats occurred in the total amount of type II protein kinase with no change in type I. These changes could be related to the hypertrophy and hyperplasia of the thyrotrophs in a similar manner as that described for TSH and the thyroid. The alterations in type II protein kinase could be related to the thyrotroph cell cycle, more specifically with entrance into S phase. The increase in type II found in rats at 14 days contrasts to the decrease in type II found in chickens at 14 days. This could simply reflect measurements taken in a fluctuating pattern of type II protein kinase. It is also possible that the decline in type II protein kinase seen in the chicken adenohypophysis is a direct consequence of reduced $T_3$, $T_4$ plasma levels. It is well known that the adenohypophysial somatotrophs are trophically maintained by thyroid hormone and upon removal of $T_3$ and $T_4$, the growth hormone content of the adenohypophysis rapidly declines following cellular atrophy (177, 178). This would be similar to the decreased type II found in the thyroid following hypophysectomy. The heterogeneity of the adenohypophysis makes it extremely difficult to be sure that observed biochemical changes are due to any specific cell type.
The observed alterations in ODC, cyclic AMP-dependent protein kinase activity ratio, and type II protein kinase observed in adenohypophysial hypertrophy and hyperplasia are similar to the enzyme changes seen during thyroid growth. It is likely that these two tissue growth responses are fundamentally the same and express the same biochemical events that have been demonstrated to occur in the growth of cells in culture. In conclusion, the coordinated events of ODC induction with subsequent polyamine synthesis and rRNA accumulation together with the specific phosphorylation of the functional units controlling translation and transcription can be initiated via cyclic AMP-dependent protein kinase by a hormone binding to a membrane receptor and activation of adenylate cyclase.

Experiments with triiodothyronine and aminophylline indicate two general mechanisms in the transcriptional induction of hepatic ODC. Ornithine decarboxylase was induced maximally by a dose of $T_3$ (300 μg/kg) known to be the most effective in relation to the incorporation of radio-labeled precursors into RNA and protein (179). There was no early increase in the activity ratio of cyclic AMP-dependent protein kinase in response to $T_3$ administration suggesting that $T_3$ induction of ODC was independent of cyclic AMP mediation. This is in line with the known direct nuclear action of $T_3$ by occupation of specific receptors (128, 180).

A cyclic AMP-mediated transcriptional induction of ODC occurs after the administration of cyclic AMP analogues and/or phosphodiesterase inhibitors in rat liver (47, 181-183). This study has substantiated the activation of cyclic AMP-dependent protein kinase
and induction of ODC in response to aminophylline and has further shown that the induction when both $T_3$ and aminophylline were administered simultaneously was additive.

It has been reported previously that growth hormone administration results in a similar induction of ODC in the liver as that described after $T_3$ or aminophylline administration (142, 143, 184). Since thyroid hormones have been implicated in the regulation of growth hormone content in the anterior pituitary, it was important to demonstrate that the effect of $T_3$ on the induction of ODC in rat liver was not due to an altered level of circulating growth hormone. Pre-treatment of rats with an amount of somatostatin that markedly decreases circulating growth hormone levels (185) had no effect on the ability of $T_3$ to induce ODC within 4 hr. The enhanced response of somatostatin-pretreated rats to $T_3$ may involve alterations in glucagon or insulin levels which may occur following initial inhibition.

Phosphodiesterase inhibitors as well as $T_3$ have been shown to enhance liver DNA synthesis preceded by general hepatocyte hypertrophy involving increased protein and ribosomal RNA (186, 187). $T_3$ as well as dibutyril cyclic AMP plus theophylline administration leads to enhanced RNA polymerase I activity, ribosome synthesis, and nucleolar enlargement 15 hr following administration. Simultaneous administration of $T_3$ plus cyclic AMP and theophylline results in an approximate additive enhancement of the above parameters (188). The additive effect of aminophylline and $T_3$ on ODC in this study is consistent with their effects on RNA synthesis. This is particularly interesting in
light of the close correlation between polyamine synthesis and ribosomal RNA accumulation in many growth systems.

Whereas the reported effects of T$_3$ plus dibutyryl-cyclic AMP and theophylline are additive in respect to RNA synthesis, their effect on DNA synthesis is clearly that of potentiation. Triiodothyronine or dibutyryl-cyclic AMP and theophylline elevate DNA synthesis measured at 20 hr by 5- and 3-fold, respectively, but 20-fold when administered together (188).

Therefore, two major routes of ODC induction in rat liver have been substantiated. One involves a direct nuclear induction brought about by T$_3$ and the other involves a cyclic AMP-dependent protein kinase-mediated mechanism characteristic of membrane active peptides or agents affecting cyclic AMP metabolism. This mechanism could involve a cytoplasmic to nuclear translocation of a cytosol protein kinase and subsequent phosphorylation of specific nonhistone chromosomal proteins involved in expression of the ODC gene as has been previously postulated (39, 189).

That the effects of T$_3$ on ODC are not confined to the liver was shown by the demonstration that T$_3$ increases ODC activity in the kidney in a similar temporal and dose-dependent manner. It was striking that the time of the maximal activity of ODC which occurs 6 hr after T$_3$ administration in the kidney was identical to that previously demonstrated after aminophylline administration (182).

The ability of T$_3$ to increase SAMD activity, the enzyme responsible for spermidine synthesis, is similar to the effects of many steroid hormones on their target tissues. A single injection of 17β
estradiol has been shown to elevate SAMD activity 8-fold within 18 hr in the rat uterus (147). A similar elevation occurred in the rat ventral prostate following testosterone administration (3). A 2-fold increase in rat liver SAMD activity also has been reported within 48 hr following a single intraperitoneal injection of 3-methylcholanthrene or phenobarbital, agents known to cause hepatomegaly (190).

The induction of SAMD by all the agents or hormones mentioned above as well as T3 in this study was blocked by actinomycin D or cycloheximide indicating de novo enzyme synthesis. The resulting production of spermidine following the induction of SAMD likely functions to facilitate the production and/or accumulation of rRNA which is a prominent feature of T3's action on the liver as well as many other tissues.

In conclusion this investigation represents a physiological study of thyroid biosynthetic processes. It suggests that the TSH released from the adenohypophysis results in significant synchronization of key biochemical events in the thyroid. The early major intracellular regulatory events appear to be activation of cyclic AMP-dependent protein kinase and induction of ornithine decarboxylase. The ability of membrane acting hormones as well as steroid hormones and T3 to regulate ODC indicate its pivotal role in the regulation of macromolecular synthesis (Figure 34).
Figure 34. Proposed model of a general sequence of events in a trophic response.
REFERENCES


120. Adiga, P. R., P. V. N. Murphy, and L. McKenzie (1971) Biochemistry, 10:708-710.


