The Effects of Hydrocortisone and Parathyroid Hormone on Bone in the Lead-Intoxicated Albino Rat

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THE EFFECTS OF HYDROCORTISONE AND PARATHYROID HORMONE ON BONE IN THE LEAD-INTOXICATED ALBINO RAT

BY

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

May 1980
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To Dr. Louis J. Blanchet, my thesis advisor, for his patience, help, and cooperation in this investigation.

To Dr. Hal McReynolds and Dr. Patrick Toto, who gave so willingly of their time to help me.

To my mother and father, Carroll and Elena Simmons, for their many years of loving encouragement.

To my uncle, Dr. Anthony B. Traub, for his support of my educational endeavors.
VITA

Paul Steven Simmons was born in Melrose Park, Illinois, on July 17, 1951. He graduated from Fenwick High School, Oak Park, Illinois, in June, 1969.

In August of 1969, he entered the University of Notre Dame, Notre Dame, Indiana, and in May of 1973, received the degree of Bachelor of Arts with a major in Pre-Professional Studies. While in college he was a Dean's Honor List student from 1970 through 1973. During the 1971-72 academic year he served as Assistant Social Commissioner for the University's Student Union.

In August of 1973 he enrolled in the Loyola University Graduate Program in Oral Biology. During this period he was awarded a Teaching Fellowship in the Departments of Physiology and Pharmacology at the Loyola University School of Dentistry, Maywood, Illinois.

In September of 1974 the author entered the Loyola University School of Dentistry and received his D.D.S. in May of 1978. In the fall of 1978, Doctor Simmons resumed his studies in Loyola's Graduate Program in Oral Biology and began the Post-Graduate Program in Periodontics. He received an appointment as Clinical Instructor in Periodontics in June, 1979, and was awarded a Teaching Fellowship in Oral and General Pathology in August of 1979.
Doctor Simmons is a member of the American Dental Association, the Chicago Dental Society, and the American Academy of Periodontology. He has been in private practice since October, 1978.
DEDICATION

To my wife, Lynne, for all her love and understanding during our first year of marriage without which I may never have completed this investigation nor my graduate education.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
</tbody>
</table>

## Chapter

I. **INTRODUCTION AND LITERATURE REVIEW.** 1  
   A. Lead Intoxication. 1  
   B. Bone Remodeling. 2  
   C. Parathyroid Hormone and Corticosteroids. 3  
   D. Mechanism of Action of Hormones. 6  
   1. Adenyl Cyclase—Cyclic Adenosine—monophosphate System 6  
   2. Cytoplasmic Receptor—Nuclear Acceptor System 10  
   E. Statement of the Problem 12  

II. **MATERIALS AND METHODS** 14  
   A. Experimental Animals 14  
   B. Experimental Protocol. 14  
   C. Post Mortem Preparation of Study Specimens. 16  
   D. Staining Procedures. 16
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>III. RESULTS</td>
<td>18</td>
</tr>
<tr>
<td>A. Weight Data</td>
<td>18</td>
</tr>
<tr>
<td>B. Blood Chemistry Data</td>
<td>18</td>
</tr>
<tr>
<td>C. Histologic Data</td>
<td>22</td>
</tr>
<tr>
<td>IV. DISCUSSION</td>
<td>43</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>50</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>51</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1. Experimental Groups</td>
<td>15</td>
</tr>
<tr>
<td>III-1. Weights</td>
<td>19</td>
</tr>
<tr>
<td>III-2. Blood Analysis</td>
<td>21</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>Perhydropentanophenanthrene nucleus (completely saturated multicyclic structure)</td>
<td>7</td>
</tr>
<tr>
<td>I-2</td>
<td>Hydrocortisone (Cortisol)</td>
<td>7</td>
</tr>
<tr>
<td>I-3</td>
<td>Adenyl Cyclase - c-AMP System</td>
<td>11</td>
</tr>
<tr>
<td>I-4</td>
<td>Receptor - Acceptor System</td>
<td>13</td>
</tr>
<tr>
<td>III-1</td>
<td>Sodium rhodizonate stain-rib-100X</td>
<td>24</td>
</tr>
<tr>
<td>III-2</td>
<td>Sodium rhodizonate stain-head of femur-100X</td>
<td>25</td>
</tr>
<tr>
<td>III-3</td>
<td>Lead leaching from pencil markings onto tissue sections-alveolar process-100X</td>
<td>26</td>
</tr>
<tr>
<td>III-4</td>
<td>Lead leaching from pencil markings onto tissue sections-alveolar process-100X</td>
<td>27</td>
</tr>
<tr>
<td>III-5</td>
<td>S.F. counterstain-rib-100X</td>
<td>28</td>
</tr>
<tr>
<td>III-6</td>
<td>S.F. counterstain-head of femur-100X</td>
<td>29</td>
</tr>
<tr>
<td>III-7</td>
<td>Group I-saline control-rib-100X</td>
<td>30</td>
</tr>
<tr>
<td>III-8</td>
<td>Group I-saline control-rib-200X</td>
<td>31</td>
</tr>
<tr>
<td>III-9</td>
<td>Group I-saline control-condyle-100X</td>
<td>32</td>
</tr>
<tr>
<td>III-10</td>
<td>Group II-PTH-rib-100X</td>
<td>33</td>
</tr>
<tr>
<td>III-11</td>
<td>Group II-PTH-rib-200X</td>
<td>34</td>
</tr>
<tr>
<td>III-12</td>
<td>Group II-PTH-condyle-100X</td>
<td>35</td>
</tr>
<tr>
<td>III-13</td>
<td>Group III-Cortisol-rib-100X</td>
<td>36</td>
</tr>
<tr>
<td>III-14</td>
<td>Group III-Cortisol-rib-100X</td>
<td>37</td>
</tr>
<tr>
<td>III-15</td>
<td>Group III-Cortisol-rib-200X</td>
<td>38</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>III-16</td>
<td>Group III-Cortisol-condyle-100X</td>
<td>39</td>
</tr>
<tr>
<td>III-17</td>
<td>Group IV-PTH + Cortisol-rib-100X</td>
<td>40</td>
</tr>
<tr>
<td>III-18</td>
<td>Group IV-PTH + Cortisol-rib-200X</td>
<td>41</td>
</tr>
<tr>
<td>III-19</td>
<td>Group IV-PTH + Cortisol-condyle-100X</td>
<td>42</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

A. LEAD INTOXICATION

Lead poisoning usually results from the ingestion of lead-containing substances such as water which has stood in lead pipes, lead-base paint, and objects containing lead solder or from the inhalation of fumes containing volatilized lead from burning storage batteries. Other means which inadvertently lead to lead poisoning include the imbibing of illicit ("corn") whiskey contaminated by lead solder in the pipes of the still or victims surviving gun-shot wounds who retain the embedded pellets (bullets or buckshot) which can cause poisoning many years later after entrapment in a serous cavity. As a rule, absorption by any route is slow, and prolonged exposure is necessary for the development of symptoms.

According to Bennett and Poskanzer (1970), lead is an example of a cumulative poison which is slowly excreted and is almost never seen or described as an acute poison. Symptoms develop slowly, but mostly after chronic exposure. Lead which is absorbed is deposited in bones as elemental lead and is metabolized in a manner similar to that of calcium. Under such chronic conditions, blood, urine, and feces levels are very low. According to the aforementioned physicians, once the source of the abnormal absorption of lead has been terminated,
virtually all of the lead in the body is directed into bone. They finally note that it requires approximately twice the time to excrete a given burden of lead as it does to accumulate it.

B. BONE REMODELING

Krook, Shively, Duncan, and Pond (1973) agreed with Whalen, Winchester, Krook, Dische, and Nunez (1971) in their observations on lead intoxicated animals in regards to the remodeling of bone that the physiological resorption, specifically of metaphyseal trabeculae, occurred mainly by osteocytic osteolysis, a pathologic-physiologic process reviewed by Belanger (1969). Osteolysis is defined as an internal resorption of bone centered around the activity of deep-seated osteocytes. It has been shown repeatedly by Belanger (1965, 1968, 1970), Belanger, Semba, Tolnai, Copp, Krook, and Gries (1966), Brown, Krook, and Pond (1966), and Henrikson (1968) that osteolysis is the primary mechanism in bone resorption, both under physiologic conditions and in diseases characterized by excessive resorption.

This deep seated bone-resorption has been related by some authors to the death of the osteocyte. Most of them have interpreted this phenomenon as passive. Pritchard wrote in 1956 "the osteocyte is an essential constituent of bone, for when the cells die, the matrix around them, although persisting for a time, eventually crumbles and is removed to be replaced by living bone".

Rutishauser and Majno (1951) have recognized perilacunar resorption as a result of cellular activity. They called it "physiological necrosis". These authors described the phenomenon as occurring "at the
center of the trabeculae" in spongy bone and "at the periphery of the Haversian systems" in compact bone. The dying cells often show a "splitting nucleus" and "reappearance of alkaline phosphatase".

Whalen, et al., (1971) and Krook, et al., (1973) concluded that the initial bone lesion in these animals was the result of the inhibition of osteocytic activity resulting in a widening of the trabeculae, eventual necrosis of osteocytes with the now dead bone tissue becoming the object of osteoclasia. Osteoclasia is a secondary phenomena involved in the removal of already altered bone such as occurs in necrosis from fractures or from bone altered by osteolysis (Belanger, Semba, Copp, Tolnai, Krook, and Gries, 1966).

C. PARATHYROID HORMONE AND CORTICOSTEROIDS

While the physiological and pharmacological effects of parathyroid hormone (PTH) and hydrocortisone (cortisol) on bone have been documented and are thus well known, their precise mechanisms of action remain elusive and have yet to be illucidated after more than three decades of intense investigation (Albright and Reifenstein, 1948; Milne, 1951; Dent, 1953; Sissons and Hadfield, 1955; Silberberg and Silberberg, 1956; Talmage, 1956; Bernick and Ershoff, 1963; Arnaud, 1967; Copp, 1970; Burns, 1974; Daughaday, 1975; Guyton, 1976).

Parathyroid hormone (PTH) controls body fluid calcium levels principally by regulating the removal of calcium from bone. After PTH administration, marked elevations in plasma calcium levels occur within several hours (Talmage, 1969). PTH stimulates osteoclast activity in vitro (Holtrop, Raisz, and Simons 1974), increases osteoclast populations
in vivo (Tatevossian, 1973), and accelerates the rate of skeletal re-modeling (Heller, McLean, and Bloom, 1950).

Systemically, corticosteroids affects a wide variety of metabolic processes. The deleterious affect they have on bone (Winter, Silberberg, and Stoerk, 1950; Pollis, 1951; Sissons and Hadfield, 1955; Silberberg and Silberberg, 1956; Laron, Muhlethaller, and Klein, 1958; Bernick and Ershoff, 1963), ground substance (Seifter, Ehrich, Boeder, Buth, and Hauser, 1953; Sobel, Gabay, and Johnson, 1958), and fibroblastic activity (Baker and Whitaker, 1950; Sissons and Hadfield, 1951; Gerarde and Jones, 1953), and their inhibition of the inflammatory reaction are well documented.

Roberts in 1969 and Kenner in 1970 found that the effect of cortisol on bone is dose and time dependent. Low doses of cortisol (1-5 mg/kg/day) stimulate bone resorption and high doses of cortisol (20-75 mg/kg/day) inhibit bone resorption. The low doses of cortisol reduced the bone volume by increasing the number of osteoclasts and decreasing the number of osteoblasts. Over a long time period any dosage over 2.0 mg/kg/day inhibits bone resorption (Roberts, 1969). In these experiments Roberts also noted that at low doses the corticosteroid action is characterized by increased progenitor cell proliferation, increased number of osteoclasts, and increased resorption of alveolar bone. At the higher dose levels, progenitor cell proliferation and bone formation were depressed without any evidence of increased resorption of the alveolar bone. These findings suggest two antagonist mechanisms; 1) direct inhibition of progenitor cell proliferation by
cortisol and 2) inducement of a secondary hyperparathyroidism by cortisol which results in increased bone resorption.

Corticosteroids reduce serum calcium levels and also have an antiproliferative action on cells. It has been suggested that the increased numbers of precursor cells and osteoclasts is a result of the increase in PTH secretion (Talmage, 1969) caused by the lowered serum calcium after the administration of cortisol (Storry, 1960; Stoerk, Peterson, and Jelinek, 1963; Gordan, Hansen, and Lubick, 1967; Rasmussen, 1968).

Roberts also states that the high dose levels of corticosteroid blocks the parathyroid mediated effect (the increased osteoclasts and bone resorption) by inhibiting parathyroid hormone-stimulated proliferation of fibroblasts. It is also possible that high doses of cortisol could act directly upon the parathyroid glands and depress the secretion of PTH.

In an early study of the interrelationship between cortisone and parathyroid extract in rats, Laron, Muhlethaler, and Klein (1958) generally summarized that an excess of parathyroid hormone caused changes in teeth and bone, and it also induced hypercalcemia and nephrocalcinosis. They further stated that cortisol also produced changes in bone, but those changes manifested were different from those induced by an excess of parathyroid hormone. Cortisol produces hypercalcuria without causing hypercalcemia, and, according to Pincus, Natelson, and Lugovoy (1951), it may even reduce normal concentrations of serum calcium; it has been used to reduce elevated serum calcium levels in hypervitaminosis D (vitamin D poisoning), infantile hypercalcemia, and sarcoidosis.
On the other hand, Dent (1956) claimed that cortisone was ineffective in lowering the hypercalcemia of hyperparathyroidism in man.

Parathyroid hormone is a polypeptide with a molecular weight of approximately 8500 whose main and perhaps only function is the maintenance of calcium homeostasis. On the other hand, hydrocortisone is classified as a steroid with a structural nucleus common to all derivatives of this group: the perhydropentanophenanthrene structure, with a molecular weight of approximately 232.84, being essentially derived from a cholesterol precursor (Figures I-1, I-2).

Unlike parathyroid hormone, whose release is primarily determined by perfusing glandular serum levels of calcium, it is now known that cortisone and hydrocortisone are regulated by a series of feedback mechanisms involving other endocrine and associated structures: the hypothalamus and pituitary gland. The one thing they do have in common is their initial reaction with a specific receptor located in the membrane as is the case of the former (Rasmussen, 1970, 1971) or in the cytoplasm by the latter (Burns, 1974). In both cases, the receptor sites tend to have a conformational structure complementary to the structure of the hormone allowing it to combine or conjugate to the former with a high degree of affinity. It, therefore appears that two major systems exist which tend to help explain most hormonal action mechanisms with the exception of insulin and thyroid hormone whose effects on metabolic activity in practically all cells remain obscure.

D. MECHANISM OF ACTION OF HORMONES

1. Adenyl Cyclase - Cyclic Adenosine Monophosphate System
Figure I-1  Perhydropentanophenanthrene nucleus
(completely saturated multicyclic structure).

Figure I-2  Hydrocortisone (Cortisol)
The nucleotide adenosine 3',5'-monophosphate (c-AMP) is now recognized as the key mediator in the action of many processes in the animal and plant kingdoms. It was largely through the patient, painstaking investigations of Sutherland and his coworkers over the last 20 years, summarized in a lecture delivered when he received the Nobel Prize in Physiology in Stockholm, Sweden, in December, 1971, that heralded the rivalry of c-AMP to adenosinetriphosphate (ATP) in the biological world (Sutherland, 1972). Since that time, investigations on the role of c-AMP have developed enormously. Not only is c-AMP found in the cells of all animal tissues studied, but it has been recently identified in bacteria as a necessary element for the induction of certain enzymes. It has also been identified as an attractant substance for such organisms as amoeba and the slime mold causing them to aggregate.

In 1957 Sutherland and Rall reported that cyclic AMP mediates the effects of epinephrine on liver phosphorylase. Subsequent studies (Robinson, Butcher, and Sutherland, 1968) have shown that the physiological actions of many other hormones and biologically active substances such as corticotrophin, thyrotrophin, serotonin, vasopressin, and luteinizing hormone are also mediated by this nucleotide. Interaction of these substances with their respective target cells leads to activation of adenyl cyclase, a membrane-bound enzyme that catalyzes the conversion of adenosine triphosphate to cyclic AMP. The cyclic AMP produced affects other cellular processes, such as activation, induction, or secretion of enzymes, active transport of ions across cellular membranes, or release of other hormones. Activation of the particular system involved causes
the physiological response attributed to the hormone. In many instances the intracellular process stimulated by cyclic AMP is still unknown (Aurbach, Potts, Chase, and Melson, 1969).

Parathyroid hormone causes a marked increase in activity of adenyl cyclase from renal cortex (Chase and Aurbach, 1968), isolated renal cortical tubules (Melson, Chase, and Aurbach, 1968), fetal rat calvaria and iliac bone from mature rats (Chase, Fedak, and Aurbach, 1969). Significant stimulation of the enzyme can be detected within 15 seconds after adding PTH to the test systems previously mentioned. This change is the earliest response to PTH that has been observed, and the enzyme in isolated tubules from renal cortex is sensitive to the hormone at very low concentrations (0.05 μg/ml).

Certain hormones activate adenyl cyclase in brain, adrenal, thyroid, and skeletal muscle but not in bone and kidney. In contrast, parathyroid hormone causes stimulation of the enzyme only in the latter tissues (Chase, Fedak, and Aurbach, 1969). Parathyroid hormone affects adenyl cyclase only in tissues where it has a known physiological function.

It is apparent from data presented by Aurbach, Potts, Chase, and Melson in 1969 that cyclic AMP is a mediator in the mechanism of action of parathyroid hormone and that the metabolic processes leading to the observed physiological effects of the hormone on both bone and kidney are initiated by a common mechanism: stimulation of adenyl cyclase. The exact intracellular metabolic processes affected by cyclic AMP in renal and skeletal tissue are unknown.

The manner in which c-AMP mediates hormonal activity appears to
be constant for parathyroid hormone in all mammalian species studied so far. It has been dubbed a "second messenger". Visualize PTH as the first messenger, circulating in the plasma, encountering and subsequently interacting with a specific receptor in the plasma membrane of the target cell. The receptor [possibly linked to or an integral part of the enzyme adenyl cyclase (AC)], which imparts specificity, interacts with PTH, thus activating AC, which in turn catalyzes the formation of c-AMP from ATP. The newly formed c-AMP then activates or triggers the response characteristic of and recognized as the biological action of PTH. As a part of nature's "checks-and-balances", or homeostatic mechanism, the activity of c-AMP is regulated by a potent enzyme known as phosphodiesterase. This cyclic nucleotide phosphodiesterase, found in the particulate and soluble fractions of the cell, catalyzes the hydrolysis of cyclic AMP to adenosine 5'-monophosphate. Inhibition of the phosphodiesterase also causes increased intracellular concentration of cyclic AMP, but so far no hormone has been found to be an inhibitor of this enzyme (Aurbach, Potts, Chase, and Melson, 1969). A schematic representation of this second messenger may be seen in Figure 1-3 (modified after Sutherland, 1972).

2. Cytoplasmic Receptor-Nuclear Acceptor System

According to Burns (1974), all of the steroid hormones exert their metabolic effects, more or less, through this system. It is hypothesized that these steroids are capable of penetrating the cell membrane and combining with a highly specific protein receptor in the cytoplasm. This conjugated steroid-receptor complex, in turn, is translocated to the
Figure I-3 Adenyl Cyclase - c-AMP System

Stimulus

Parathyroid Glands

Released PTH (First Messenger)

PTH

PTH Receptor Site

Cell Membrane

Kinase

C-AMP Kinase Complex

Triggers Physiologic Response of PTH

Inactivation of C-AMP by Phosphodiesterase

Adenyl Cyclase
nucleus which enters to then combine with specific acceptors located on chromatin. This complex steroid-receptor-acceptor now causes nuclear genetic material to produce messenger ribose nucleic acid (m-RNA), which now signals and directs the fabrication of some highly specific enzyme whose action is directly responsible for producing the physiologic expression of the hormone's activity. Specificity of this system resides in the conformational structure of the receptor protein to the steroid hormone and this complex condensing to the nuclear chromatin acceptor. This system may be visualized in Figure I-4 (modified after Burns, 1974).

E. STATEMENT OF THE PROBLEM

Since a number of investigations (previously cited) have been reported where varying doses of PTH and cortisol were used over rather extended periods of time it seemed reasonable to conduct a short-term study using relatively high doses of the two hormones, but administering them at broader time intervals. In other words, all test animals to be studied would be already rendered lead-intoxicated before they would be challenged by PTH, cortisol, and a combination of the two, and at some subsequent point in time, sacrificed. Bone tissue sections and blood samples will be examined in order to determine whether or not any changes occurred during the acute experimental period that might parallel those of the aforementioned chronic studies.
Figure I-4  Receptor-Acceptor System

Steroid Hormone

Cell Membrane

Steroid-Receptor

Complex

Receptor

Nuclear Membrane

Steroid-Receptor

Complex

Acceptor

Chromatin

m-RNA

Enzyme Synthesis

Physiologic Expression Attributed to Hormone
CHAPTER II

MATERIALS AND METHODS

A. EXPERIMENTAL ANIMALS

Twenty-eight, young, adult, male, Sprague-Dawley rats, whose weights ranged from 174 to 311 grams, were used in this investigation. Upon receipt, the animals were allowed to become acclimated to their new environment and fed a normal diet of Purina Fox Chow pellets with water allowed ad libitum.

Following this seven day acclimation period, the animals were then maintained on a diet consisting of ground Purina Chow meal, and again a free intake of water. Lead (Pb) intoxication was induced by the addition of 1000 parts per million of the acetate \[ \text{Pb(COOCH}_3\text{)}_2 \]; per cent Pb = 63.71; Aldrich Chemical Co.] to the meal. Calcium and phosphate carriers were also added to the diet in 1.1% and 1.0% amounts respectively. All animals were maintained on this diet for the entire two week experimental period. The animals were then divided into four groups and kept on the aforementioned lead, calcium, and phosphate diet for a period of seven days.

B. EXPERIMENTAL PROTOCOL

The treatment for the four groups was as follows:

1. One animal was selected from each group immediately prior to the administration of the test drugs for
blood samples in order to determine pre-injection levels of calcium, phosphate, and lead. Blood samples (10 ml each) were obtained by cardiac puncture and the Ca\(^{++}\) and Pi were determined by SMA-12/80, and the Pb by the dithizone urinalysis method modified for blood analysis (Race, 1978). Those rats surviving were no longer used in this investigation.

2. Exactly 1 week following placement of the animals on the lead-intoxicating diet, on which they were maintained through the remainder of the study, injections were begun:

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>6</td>
<td>Controls - 2.0 ml 0.9% saline</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>Parathyroid Hormone (Lilly) - 2.0 ml (1 ml = 100 USP units)</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>Hydro-Cortisone acetate (Cal-Biochem-Behring) - 2.0 ml (1 ml = 2.5 mg)</td>
</tr>
<tr>
<td>Group IV</td>
<td>6</td>
<td>1 ml Parathyroid Hormone plus 1 ml Hydro-Cortisone acetate</td>
</tr>
</tbody>
</table>

All drugs were administered via intraperitoneal injection on days 1, 3, 5, and 7. Each animal was weighed on day 1 before the administration of the test drugs for its group and at the end of day 8 which marked the termination of
the experimental period. Prior to sacrificing, one animal was selected from each group from which blood samples were again obtained for Ca++, P_i, and Pb determinations.

C. POST MORTEM PREPARATION OF STUDY SPECIMENS

Sacrificing consisted of etherization in a large glass desiccator followed by pneumothorax. Immediately following sacrificing, surgical dissection was used to obtain tissue samples:

1. Alveolar process - buccal to mandibular first molar
2. Condyle of the mandible
3. Fourth rib
4. Femur (head)

All soft tissue attachments were manually removed, and the formentioned specimen placed in 10% neutral formalin for fixation and storage for a period of 90 days. Following fixation, the specimens were rinsed several times with distilled water and then placed in a 5% H_2SO_4 solution containing 5% Na_2 SO_4, for decalcification (Gomori, 1952) for a period of 3 weeks. The decalcified samples were removed from this solution, rinsed with distilled water and then embedded in paraffin for sectioning and staining.

D. STAINING PROCEDURES

a. Hematoxylin and eosin

b. Rhodizonate (method for lead salts)

Sections were placed in a 0.2% solution of sodium rhodizonate (Sigma) in 1% acetic acid for 60 minutes, then counter stained with 0.1% light green S.F. in 1% acetic acid for 30 seconds.
All sections were permanently mounted and subjected to light microscopic study and evaluation which is reported and discussed in subsequent chapters.
CHAPTER III

RESULTS

A. WEIGHT DATA

Upon receiving the twenty-eight experimental animals they were divided into four groups, each group containing seven rats. After the one week acclimation period the rats were placed on the experimental diet as described in Chapter II: Materials and Methods. At the end of seven days, each rat was weighed, blood samples were taken from one rat in each group, and the administration of the experimental drugs was begun. Sacrificing took place at the end of one week and prior to this weight determinations were again made on the animals. Pre and post-injection weights are recorded in table III-1.

It can be seen that the animals in Group I (saline administered controls) experienced an average gain in weight of 11.5 grams. Rat no. 5 was the only animal in this group to lose weight, and this amount was negligible (1 gram). The rats in Group II who received 200 USP units of parathyroid hormone every other day only realized an average weight gain of 3.8 grams. The experimental animals in Group III receiving 5 mg of hydrocortisone per injection and Group IV, the parathyroid hormone and hydrocortisone group, experienced average weight losses of 2.5 and 23.5 grams respectively.

B. BLOOD CHEMISTRY DATA

Blood samples (10 ml each) were taken from one rat in each group
Table III-1

Weight (grams)

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat 1 pre</th>
<th>Rat 1 post</th>
<th>Rat 2 pre</th>
<th>Rat 2 post</th>
<th>Rat 3 pre</th>
<th>Rat 3 post</th>
<th>Rat 4 pre</th>
<th>Rat 4 post</th>
<th>Rat 5 pre</th>
<th>Rat 5 post</th>
<th>Rat 6 pre</th>
<th>Rat 6 post</th>
<th>Rat 7 pre</th>
<th>Rat 7 post</th>
<th>Average gain/loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (saline)</td>
<td>204</td>
<td>229*</td>
<td>266</td>
<td>278</td>
<td>240</td>
<td>253</td>
<td>259</td>
<td>273</td>
<td>311</td>
<td>310</td>
<td>227</td>
<td>233</td>
<td>269*</td>
<td>-</td>
<td>+ 11.5</td>
</tr>
<tr>
<td>Group II (PTH)</td>
<td>233</td>
<td>245*</td>
<td>240</td>
<td>261</td>
<td>228</td>
<td>244</td>
<td>263</td>
<td>252</td>
<td>248</td>
<td>223</td>
<td>298</td>
<td>308</td>
<td>174*</td>
<td>-</td>
<td>+ 3.8</td>
</tr>
<tr>
<td>Group III (Hydrocortisone)</td>
<td>234</td>
<td>262*</td>
<td>269</td>
<td>246</td>
<td>208</td>
<td>207</td>
<td>209</td>
<td>211</td>
<td>229</td>
<td>221</td>
<td>229</td>
<td>216</td>
<td>275*</td>
<td>-</td>
<td>- 2.5</td>
</tr>
<tr>
<td>Group IV (PTH + Hydrocortisone)</td>
<td>210</td>
<td>194*</td>
<td>256</td>
<td>242</td>
<td>260</td>
<td>235</td>
<td>203</td>
<td>170</td>
<td>268</td>
<td>244</td>
<td>251</td>
<td>222</td>
<td>295*</td>
<td>-</td>
<td>- 23.5</td>
</tr>
</tbody>
</table>

* Rats used for blood samples via cardiac puncture (see table III-2)
at random prior to the experimental injections, and then prior to sacrifice. The data collected from these animals (indicated in table III-1) selected for serum analyses of calcium (Ca$$^{++}$$), inorganic phosphate (P$_i$), and lead (Pb) can be visualized in table III-2.

A marked difference can be seen between the pre and post-injection blood levels of Pb: pre-injection levels averaged 9.2 µg per 100 ml serum, while post injection levels averaged 36.4 µg per 100 ml serum; a difference of 27.2 µg %.

Pre-injection levels of calcium in all the experimental animals averaged 11.88 mg per 100 ml serum. The serum calcium level of the animal in Group I remained, for all practical purposes, unchanged (averaged 10.8 mg %) throughout the whole of the experimental period. The rat from Group II demonstrated a slightly elevated calcium level from 12.5 mg per 100 ml serum to 13.85 mg per 100 ml serum (averaging the two post-injection measurement levels) representing a slight gain of 1.35 mg %. The rat from Group III (hydrocortisone), however, showed an increased serum calcium level of 2.6 mg % which was 1.25 mg per 100 ml of serum greater than the final value obtained from the PTH injected animal. Finally, the measured values obtained from the Group IV rat were as follows: pre-injection = 13.0 mg %; post-injection = 16.88 mg %. This demonstrates a difference of 3.88 mg per 100 ml of serum which proved to be 3.02 mg greater than the Group II rat and 3.45 mg greater than the Group III rat (post-injection levels).

Pre-injection inorganic phosphate levels of all animals averaged 4.6 mg per 100 ml of serum. The pre and post-injection levels of P$_i$
Table III-2

Blood Analysis
($\mu g \% \text{Pb} - mg \% \text{Ca}^{++} \text{ and } \text{P}_4$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pb</th>
<th>Ca$^{++}$</th>
<th>P$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
</tr>
<tr>
<td>I</td>
<td>10.5</td>
<td>30.5</td>
<td>11.2</td>
</tr>
<tr>
<td>(Saline)</td>
<td>8.6</td>
<td>38.3</td>
<td>12.5</td>
</tr>
<tr>
<td>II</td>
<td>5.5</td>
<td>32.6</td>
<td>10.8</td>
</tr>
<tr>
<td>(Hydrocortisone)</td>
<td>12.2</td>
<td>44.2</td>
<td>13.0</td>
</tr>
</tbody>
</table>
for the Group I rat remained essentially unchanged; 3.8 mg % and 3.3 mg % respectively. The Group II rat showed a difference of 4.5 mg %, representing a loss of phosphate when compared to the pre-injection level, which was 2.23 mg % less than that of Group I. The Group III rat showed little or no change from its pre-injection level of 3.9 mg per 100 ml serum to its post-injection level of 4.15 mg per 100 ml serum, a difference of only +0.25 mg %. Finally, the Group IV (PTH-cortisol) rat, whose pre-injection serum Pi level was 5.2 mg % realized a post-injection 4.2 mg % difference to 1.02 mg per 100 ml of serum.

C. HISTOLOGIC DATA

Lead intoxication as demonstrated by the high blood levels was verified by the red staining of the insoluble lead salts intranuclearly with the sodium rhodizonate (Figures III-1 and III-2). The strength of staining may be compared to Figures III-3 and III-4 where the lead of the pencil tip markings identifying the slide were allowed to leach onto the tissue sections during the staining process. The resultant specimens stained a deep scarlet red. The light green S.F. counterstain completely masked the rhodizonate staining of the lead salts as demonstrated in Figures III-5 and III-6.

The hematoxylin and eosin stained sections of alveolar process and head of the femur were unremarkable. However, the rib and condyle histologic sections showed some differences between the four groups.

The PTH injected animals (Group II) showed a widening of the band of calcifying cartilage and zone of hypertrophy. There was also an increase in the number of cartilage columns and an increase in the marrow
space cellularity with a concomitant decrease in the amount of calcification and ossified structures. This may be visualized in Figures III-10, III-11, and III-12. The lead intoxicated saline controls by comparison appear in Figures III-7, III-8, and III-9. Cortisol therapy in the Group III rats (Figures III-13, III-14, III-15, and III-16) caused a marked thinning of the proliferative (calcifying) and hypertrophic cartilage in the rib and condyle. The cartilage columns appeared encased in the ossifying matrix. There was also decreased ossification. In Group IV where the rats received PTH and cortisol the sections approached a medium between the controls and the PTH group. There was less calcification and there was a slight increase in the marrow space cellularity as compared to the controls. These changes are illustrated in Figures III-17, III-18, and III-19.
Figure III-1 Sodium rhodizinate stain - rib

100x
Figure III-2  Sodium rhodizonate stain - head of femur
Figure III-3  Lead leaching from pencil markings onto tissue sections - alveolar process

100X
Figure III-4  Lead leaching from pencil markings onto tissue sections - alveolar process
Figure III-5  S.F. counterstain - rib
Figure III-6  S.F. counterstain - head of femur

100X
Figure III-7  Group I - saline control - rib

100X
Figure III-8  Group I - saline control - rib

200X
Figure III-9  Group I - saline control - condyle

100X
Figure III-10  Group II - PTH - rib

100X
Figure III-11  Group II - PTH - rib

200X
Figure III-12  Group II - PTH - condyle

100X
Figure III-13  Group III - Cortisol - rib
Figure III-14  Group III - Cortisol - rib

100X
Figure III-15  Group III - Cortisol - rib

200X
Figure III-16  Group III - Cortisol - condyle

100X
Figure III-17  Group IV - PTH + Cortisol - rib

100X
Figure III-18  Group IV - PTH + Cortisol - rib

200X
Figure III-19  Group IV - PTH + Cortisol - condyle

100X
CHAPTER IV

DISCUSSION

While the initial thrust of this investigation was to study the effects of cortisol and parathyroid hormone (PTH), alone and in combination with each other, on bone in the lead intoxicated albino rat, two areas, initially assigned minor importance, surfaced with some interesting results. One area deals with the weight of the animals, and the other is related to serum levels of calcium ($\text{Ca}^{++}$), phosphate ($\text{P}_i$), and lead ($\text{Pb}$).

Data reflecting weight changes is reported in Table III-1, where the controlled Group I demonstrated a mean increase in weight of 11.5 grams. This represents only a 4.4% increase over their initial weights. Group II, those animals receiving PTH, showed a weight gain of only 1.5% of their initial mean value; their actual mean increase was 3.8 grams. In terms of weight gain or loss, Groups III and IV fared worse than those in Groups I and II, in fact the former lost weight. Group III, those receiving cortisol, lost an average of 2.5 grams, which is not considerably great when compared to 23.5 gram loss demonstrated by Group IV, who received the combined PTH-cortisol injections. The data from Groups III and IV is not inconsistent with that reported in the literature. According to Bernick and Ershoff (1963), the administration of cortisone to rats results in a reduction in food intake by
these animals, and negative nitrogen balance. Earlier, Bavetta, Bernick, and Ershoff (1959) maintained rats on a qualitatively adequate diet which was limited to approximately 5 grams per day. They were able to show that these rats fed this low caloric diet did not necessarily lose weight, but had their weights plateau.

Comparatively, the animals in Groups I and II (saline controls and PTH injected rats, respectively) did show a little gain in weight of +11.5 grams and +3.8 grams respectively. They were allowed food and water freely, and should have shown greater weight gains than those observed. However, it should be reiterated at this point that their ground Purina Fox Chow food was adulterated with lead acetate (Pb=1000 ppm), 1.1 percent Ca, and 1.0 percent Pi after the manner of Hsu, Krook, and Pond (1973). The ingestion of the heavy metal Pb might account for this almost catabolic effect with respect to their weights. One of the numerous symptoms of chronic lead poisoning is anorexia, which perhaps helps to explain the lack of weight gain in the first two groups.

Upon analyzing the data obtained from pre and post-injection blood samples, tested for Ca++, P1, and Pb, it was of interest to note that the pre-injection blood levels of Ca++ and P1 were all within their normal ranges (Ca++, mean = 11.8 mg/100 ml serum; P1, mean = 4.8 mg/100 ml serum). On the other hand, pre-injection Pb values appeared to be relatively inconsistent (see Table III-2) with a mean value of 9.2 μg/100 ml serum which, according to Berkow (1977), appears to be insignificantly low, and relative to human beings, low enough not to be toxic (normal non-toxic human level 15 to 40 μg %). While these values appear
to be low, it should be pointed out that these animals had only been exposed to the lead in their diet for a period of only one week, a time period certainly not long enough to be considered toxic. However, at the end of the experimental period, the serum Pb values had increased by some 76 percent over their original values, putting the animals at the lower limits of chronic lead intoxication. In the beginning of this study, it appears obvious that the low borderline toxic levels of Pb had little or no effect on serum Ca\textsuperscript{++} or P\textsubscript{i} levels in all groups, especially Group I rats. Group II animals receiving PTH demonstrated a rise in serum Ca\textsuperscript{++} of 1 to 2 mg over their pre-injection levels, but a fall of approximately 4.5 mg of P\textsubscript{i} compared to the pre-injection levels. These effects of parathyroid hormone on Ca\textsuperscript{++} and P\textsubscript{i} are well known and consistent with the literature with the exception of the very slight hypercalcemia, observed in this group, which is believed to be due to suppression of mobilization of Ca\textsuperscript{++} from bone due to the presence of low, toxic Pb concentrations.

The animals in Group III receiving cortisol demonstrated a rise in serum Ca\textsuperscript{++} to the order of 2.85 mg %, representing a 20 percent increase over their pre-injection level. However, there was no appreciable difference between pre-injection and post-injection levels of P\textsubscript{i}. While this data is somewhat in agreement with the literature, one cannot help but feel that the presence of Pb might have prevented some dramatic disclosures in this group of animals.

Finally, Group IV rats receiving PTH and cortisol do show some dramatic differences between pre and post-injection levels of Ca\textsuperscript{++} and
Pi. This group demonstrated a marked increase in Ca\(^{++}\) levels to the order of 22.8 percent higher than its pre-injection levels, and slightly higher than the post-injection levels of Groups II and III (3.02 mg and 3.22 mg respectively). The observation that the post-injection Ca\(^{++}\) concentration of 16.87 mg per 100 ml serum was higher in Group IV than the post-injection level of Group II which was observed to be 13.85 mg per 100 ml serum can only be explained on the basis of the presence of the Pb. According to Bentzel, Carbone, and Rosenberg (1964), Lazor and Rosenberg (1964), and Mundy and Raisz (1974) glucocorticoids have been shown to be effective in the treatment of hypercalcemia associated with hematological neoplasms, however, their mechanism of action remains undefined to any real degree of satisfaction (Strumpf, Kowalski, and Mundy 1978). The post-injection Pi level, while markedly lower than the pre-injection level (4.18 mg), was almost identical to the pre and post-injection levels of Group II.

Upon reflecting, one cannot help but to consider the presence of Pb to be a factor responsible for the results obtained relative to the weights of the animals, their serum Ca\(^{++}\) and Pi levels, and even the changes in Pb levels under the influence of PTH, cortisol, and PTH + cortisol. Earlier chronic Pb studies, ranging from 9 to 20 weeks, allowed time for the toxic effects of the heavy metal to set in, while the basic idea in this study was to have a low grade of toxicity develop over a very short period of time (2 weeks) in order to see if this "subliminal" toxicity had a greater or lesser effect in the areas under
investigation. The number of bizarre findings in this study are quite intriguing and suggests further extension of this study relative to the use of larger and varying doses of both PTH and cortisol; extension of the time of lead exposure prior to, and/or during administration of the hormones. A final avenue would include: samples of epiphyses of long bones, ribs, and sections of liver and kidney. The use of rhodizonate should not be abandoned, but the manner of decalcification of hard tissue should be further investigated due to the presence of artifacts in the specimens possibly caused by the use of sulfuric acid. As reported by Pearse (1960) the rhodizonate method is quite sensitive for lead: "In practice I have found that small concentrations of an insoluble lead salt (PbSO₄) in the tissues can readily be demonstrated by the rhodizonate method." These insoluble lead salts are transformed from soluble Pb deposits in the bone by the decalcification procedure utilizing H₂SO₄ and Na₂SO₄ (Gomori, 1952).

Histologically, the results in this experiment are consistent with past literature. Group II, the PTH injected animals, showed a decrease in the amount of calcification in growth areas (rib and condyle). As reported by Yonaga (1978) on sections of rat proximal tibia sections, the rib and condyle sections of Group II showed a widening of the cartilaginous plate and a widening of the hypertrophic cell layer. There was also increased cellularity in the marrow spaces with a decrease in ossification. Laron, Muhlethaler, and Klein (1958) reported that parathyroid extract produces changes expected from excess parathyroid hormone. There was interference with normal calcification and hypercalcemia.
The Group III histologic studies also concurred with past references (Follis, 1951; Sissons and Hadfield, 1955; Laron, Muhlethaler, and Klein, 1958; Bernick and Ershoff, 1963; Jee, Park, Roberts, and Kenner, 1973). There was a narrowing of the cartilaginous growth zones (rib and condyle) and an encasement of the cartilage columns in the ossifying matrix. There was also a decrease in the amount of calcification taking place. It seemed that the whole process of cartilage changing into mature bone was at a standstill. As Bernick and Ershoff reported in 1963, "one may conclude that cortisone, by its action upon the acid mucopolysaccharides, alters the ground substance of bone, thus inhibiting its proper calcification, its physiologic resorption and, therefore, its reorganization".

This reduction in bone activity is time and dose dependent as reported by Jee, Park, Roberts, and Kenner in 1973. Low doses of cortisol (1 and 5 mg/kg/day) reduce bone through increased bone resorption and high doses (20 and 75 mg/kg/day) depress bone resorption in rats. Also noted by Roberts in 1969 was that at the high dose levels of cortisol, progenitor cell proliferation and bone formation were depressed without any evidence of increased resorption of bone, and at low doses the corticosteroid action was characterized by increased progenitor cell proliferation, increased number of osteoclasts, and increased resorption of bone. The histology presented by the rats in Group III is consistent with the data presented by Roberts. The rats in Group III received 5 mg every other day for seven days. The average weight of the rats over the experimental period was approximately 250 grams, therefore, the
dose averaged out to 20 mg/kg/injection, which according to the previous studies would appear on the low end of the high dose level spectrum. Sissons and Hadfield (1955) reported a retardation of bone growth and organization with this "high" dose of cortisol.

When both hormones were given simultaneously the results obtained histologically demonstrated an antagonistic action between cortisol and PTH. The blood chemistry levels do not verify this but, again, this may be due to the presence of Pb which has an osteolytic effect upon bone. The dosage of cortisol plays an important role here. As reported by Roberts (1969) at low doses cortisol induces a secondary hyperparathyroidism which results in increased bone resorption. In this case, the effect of exogenous and endogenous PTH would be cumulative. At the higher doses though, the inhibitory effect of the corticosteroid takes precedence and the hormonal antagonism occurs. This resultant minimal PTH effect was evident in the Group IV rats.
SUMMARY AND CONCLUSIONS

The data obtained during this study showed a definite effect on Ca$^{++}$ and Pi metabolism as reflected by the serum studies on all of the animals treated with PTH, cortisol, and PTH + cortisol, some consistent with the prevailing literature in the presence of a lead intoxication of a very low grade. The weights of the test animals were affected likewise: although subtle (possibly due to the time and dosage parameters), histologic changes in bone also occurred. Relative to the few inconsistencies noted between the data presented in this paper and the past literature, the low lead dose and time exposure as well as the doses of the hormones administered may have had an effect. These experiments should be extended and further investigations undertaken in the manner suggested in Chapter IV.
REFERENCES


APPROVAL SHEET

The thesis submitted by Paul S. Simmons, B.A., D.D.S. has been read and approved by the following committee:

Dr. Louis Blanchet, Director
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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Date: May 12, 1980

Director's Signature: [Signature]

57