INFLUENCE OF PORCINE THYROCALCITONIN IN MAGNESIUM DEFICIENT AND NORMAL RATS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
DANIEL R. FARNELL
1969

This is to certify that the

thesis entitled

INFLUENCE OF PORCINE THYROCALCITONIN IN

MAGNESIUM DEFICIENT AND NORMAL RATS

presented by

Daniel R. Farnell

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Pathology

Charles K. Whitehair
Major professor

Date Aug 25, 1969

O-169

ABSTRACT

INFLUENCE OF PORCINE THYROCALCITONIN

IN MAGNESIUM DEFICIENT AND

NORMAL RATS

by Daniel R. Farnell

It is known that thyrocalcitonin inhibits the removal of mineral from bone and at the same time causes hypocalcemia and hypophosphatemia. On the other hand, chronic magnesium deficiency causes impaired mineralization of bones and teeth along with increased calcium deposition or concentration in soft tissues. Experiments were designed for the purpose of determining whether the administration of thyrocalcitonin would alter the manifestations of magnesium deficiency in rats.

Two experiments were performed. Weanling male rats of the Sprague-Dawley strain were used in both experiments and were fed either a magnesium-deficient or magnesium-adequate diet (control diet) *ad libitum* during the experiments.

In Experiment 1, 40 rats were divided into 2 groups and fed either the magnesium-deficient or control diet for 32 days. One-half the rats in each diet group were treated with injections of thyrocalcitonin at 12-hour intervals throughout the diet period. During the diet and treatment period, the rats were observed for the occurrence of clinical signs. At the end of the 32nd day, the rats were killed and examined for gross and microscopic lesions, and the blood plasma calcium and magnesium concentrations were determined. Thyrocalcitonin was beneficial

in that it inhibited the cutaneous hyperemia, discrete skin lesions, gingival hyperplasia, renal calcification, dentinal lesions in incisor teeth, and the hypomagnesemia that are caused by magnesium deficiency.

The effect of thyrocalcitonin in increasing the density of the metaphyseal spongy bone in the tibias was greatly enhanced in the magnesium-deficient rats. This was determined by radiographic, histopathologic, and direct examination of the tibias.

Thyrocalcitonin was detrimental in that it caused the deposition in the kidney cortex of an amorphous material located among the proximal convoluted tubules. The material stained like the amorphous intercellular substance of connective tissues, and eventually became impregnated with mineral.

Regardless of diet, thyrocalcitonin administration caused the occurrence of regularly-spaced concentric lines in the dentin of the incisor teeth, each line corresponding to a single injection of the hormone. The lines were believed to indicate an altered rate of dentin deposition or calcification.

In Experiment 2, 38 rats were divided into 2 groups and fed the magnesium-deficient or control diet exclusively for 6 days. At the end of this time, rats from each group were given single injections of thyrocalcitonin and killed by exsanguination at either 1, 3, or 5 hours after injection. The calcium and magnesium concentrations of the plasma were determined. Although there were no detectable effects of the thyrocalcitonin on the plasma magnesium concentration, magnesium deficiency caused an expected reduction in plasma magnesium. The hypocalcemic effect of thyrocalcitonin was prolonged in the magnesium-deficient rats, as compared to the nondeficient rats. This prolongation in effect of thyrocalcitonin in causing hypocalcemia is believed to be related to

the enhanced promotion of metaphyseal spongy bone density by thyrocalcitonin in magnesium-deficient rats.

Thyrocalcitonin caused definite alterations in signs and lesions of magnesium deficiency in rats. It is concluded that the metabolism of magnesium, calcium, and thyrocalcitonin are significantly interrelated.

INFLUENCE OF PORCINE THYROCALCITONIN IN MAGNESIUM DEFICIENT AND NORMAL RATS

By

Daniel R. Farnell

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pathology

1969

Dedicated to my wife, Carolyn, and to
our children: Danny, Andrea, and
the new baby, Cynthia

ACKNOWLEDGEMENTS

The thyrocalcitonin used in this research was generously provided by Dr. J. P. Aldred of the Department of Pharmacology, Armour Pharmaceutical Company.

Dr. Robert H. Dunlop of the Department of Veterinary Physiology, University of Saskatchewan, Saskatoon, Canada, gave the author useful initial information about publications on the subject of thyrocalcitonin.

The author is also indebted to many people at Michigan State University who in some way provided needed assistance in the performance of this research.

Dr. Olaf Mickelsen and Dr. Modesto G. Yang, of the Department of Foods and Nutrition, and Dr. Kenneth K. Keahey of the Department of Pathology, provided laboratory space and facilities. Drs. Delbert L. Whitenack and Glenn L. Waxler, Department of Pathology, gave advice and help in photography.

Dr. Ulreh V. Mostosky, of the Department of Small Animal Surgery and Medicine, performed the radiography of the rat tibias.

Material assistance in the handling and treatment of the rats was provided by the following veterinary students: Joe Burek, Bob Sray, and Gary Willwerth.

Dr. Vance L. Sanger, of the Department of Pathology, made useful suggestions regarding the preparation of bones for examination.

Miss Janice Fuller provided expert assistance in the typing of the manuscript.

Valuable help in the preparation of tissues for histopathologic study was provided by Mrs. Mae K. Sunderlin, Mrs. Nina L. Miller, and Mrs. Frances M. Whipple, all of the Department of Pathology.

Dr. Robert F. Langham, Professor of Pathology, was largely responsible for imparting a sufficient knowledge of histopathology so that the author felt competent in evaluating the microscopic tissue changes.

The author is grateful to Dr. C. K. Whitehair, who gave friendly, expert advice on numerous occasions, and who provided active assistance when it was needed.

The valuable influence of Dr. C. C. Morrill in providing an atmosphere in the Department of Pathology conducive to congenial relations and to the students' being able to gain significant competence in the specialty of pathology is acknowledged.

Finally, the author is grateful for financial support by the National Institute of Arthritis and Metabolic Diseases.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

INTRODUCTION

The thyroid glands of mammals, in addition to producing thyroxin and other iodinated thyronine compounds, secrete another distinct type of hormone known as thyrocalcitonin (synonym: calcitonin). Thyrocalcitonin inhibits the removal of mineral from bone, and at the same time causes a reduction in the levels of calcium and phosphate in the blood. There is evidence that thyrocalcitonin not only can inhibit the removal or resorption of mineral from bone, but also may actively promote the laying down of new bone tissue.

These effects of thyrocalcitonin are opposite to those of chronic magnesium deficiency of rats, where there is impaired mineralization of teeth, osteoporosis, hypercalcemia, and increased concentration and deposition of calcium in a number of the soft tissues.

It seemed likely that the physiological roles of magnesium, calcium, and thyrocalcitonin were significantly interrelated. Experiments were designed for the purpose of determining whether the administration of thyrocalcitonin would alter the manifestations of magnesium deficiency in rats.

Of primary interest were the occurrence in the rats of clinical signs and tissue structural changes associated both with thyrocalcitonin administration and with manipulation of the dietary magnesium intake.

Objectives

The objectives of this research were: (1) to demonstrate a significant relationship between thyrocalcitonin and magnesium metabolism; (2) to determine the prophylactic effect, if any, of thyrocalcitonin against the signs and lesions of magnesium deficiency in rats; (3) to detect possible histologic and pathologic changes in rats treated chronically with thyrocalcitonin; and (4) to extend previous studies by the author (Farnell, 1965; 1966a; 1966b; 1968) on the influence of hormones on the magnesium deficiency syndrome.

LITERATURE REVIEW

Thyrocalcitonin

Discovery. Prior to the discovery of thyrocalcitonin, it was believed that the parathyroid gland, through its effect on skeletal calcium, was primarily responsible for the control of calcium levels in the body (Copp, 1967).

A negative feedback system proposed by McLean (McLean and Urist, 1955) at the University of Chicago was considered to be a reasonable explanation of the hormonal control of calcium. According to McLean, a decrease in plasma calcium was the stimulus for the production of parathyroid hormone. Parathyroid hormone then promoted osteolysis and mobilization of calcium from the skeleton. McLean suggested that the control of hypercalcemia was due to suppression of parathyroid hormone production. The more recent work of Sanderson *et al.* (1960) cast doubt on the validity of McLean's hypothesis. Using thyroparathyroidectomized dogs, Sanderson's group found that, in dogs without parathyroid glands, the control of induced hypercalcemia was inefficient. This did not support the belief that suppression of parathyroid gland activity was responsible for the correction of hypercalcemia.

Dr. D. Harold Copp *et al.* (1962) at the University of British Columbia first recognized the existence of a hormone, which they called "calcitonin", and whose action was to lower blood calcium levels. Using dogs, Copp found that although perfusion of the functional

parathyroid-thyroid gland mass with blood high in calcium resulted in a generalized depression of blood calcium, the blood calcium increased beyond normal limits when thyroparathyroidectomy was performed. These results were evidence that some substance produced by the glands had a hypocalcemic effect. Because the thyroid and parathyroid glands were treated as a unit in their experiments, Copp *et al.* were unable to differentiate the effects of the two types of glands; and they erroneously concluded that calcitonin was produced by the parathyroid glands, rather than the thyroid glands.

The Harvard University group of P. F. Hirsch, Geraldine F. Gauthier, and P. L. Munson (1963) reported that a factor liberated from the rat thyroid gland, by hot-wire cautery *in situ* or by homogenization and extraction of the gland, was effective in causing a reduction in blood calcium. In their experiments, the parathyroid tissue was separated from the thyroid tissue, so that identification of the thyroid gland as the source of the hypocalcemic factor was possible. They named the hormone "thyrocalcitonin", following Copp's terminology for the hypocalcemic factor, with the prefix "thyro-" to indicate thyroid gland origin.

Chemical Nature. Because of its availability, thyrocalcitonin of porcine origin has been used widely in research on this hormone. It is remarkable that only about 6 years elapsed from the discovery of thyrocalcitonin until its structure had been determined and synthesis achieved. The complete amino acid sequence of both porcine and human thyrocalcitonin (Potts *et al.*, 1968; Neher *et al.*, 1968a; Neher *et al.*, 1968b) as well as synthesis of both varieties of thyrocalcitonin (Rittel *et al.*, 1968; Sieber *et al.*, 1968) was reported from the U.S.A. and Switzerland.

Both porcine and human thyrocalcitonin are single peptide chains containing 32 amino acid residues, each with a disulfide linkage between cysteine residues at positions 1 and 7 of the chain. However, there are a number of differences between porcine and human thyrocalcitonin in the amino acids found along the chains. Human thyrocalcitonin may occur as a dimer containing double the number of amino acids in the basic structure, and it seems reasonable that other examples of natural polymerization of the thyrocalcitonin molecule may exist.

Site of Production. Two primary types of secretory cells have been identified in mammalian thyroid glands. The follicular cells -- those cells that line the follicle lumen and secrete thyroxin -- are derived in embryonic life from the thyroid diverticulum, a midplane outpouching of the floor of the pharynx (Arey, 1946). The parafollicular cells (known also as interfollicular cells, "light cells", "C-cells", argyrophillic cells, etc.) have an entirely different embryonic origin; and it is these cells that produce thyrocalcitonin (Bussolati and Pearse, 1967). The paired ultimobranchial bodies, which are the origin of the parafollicular cells, are derived from the fifth (last, or "ultimate") branchial pouch. Although in almost all mammals the ultimobranchial tissue normally fuses with the thyroid gland, the ultimobranchial bodies remain distinct organs in birds, fish, amphibians, and reptiles (Copp *et al.*, 1967). Consequently, the thyroid gland of such animals as the chicken does not produce thyrocalcitonin (Kraintz and Puil, 1967); the hormone in that species is produced by the ultimobranchial bodies, and in such cases the name "calcitonin" is more appropriate (Copp *et al.*, 1967).

Physiologic Role. Bone appears to be the primary target organ of thyrocalcitonin. The effects of thyrocalcitonin and parathyroid hormone are antagonistic. Whereas parathyroid hormone promotes resorption of bone and thus hypercalcemia, thyrocalcitonin inhibits bone resorption (Aliapoulios *et al.*, 1966a) and causes a fall in blood calcium (Hirsch *et al.*, 1963).

In addition, thyrocalcitonin influences bone formation, although some of the research reports are conflicting. Milhaud and Moukhtar (1966a), using ^{45}Ca kinetic studies, concluded that thyrocalcitonin significantly decreased accretion in the rat. Using thyroparathyroidectomized rats, Baylink *et al.* (1969) obtained further, histologic evidence that thyrocalcitonin decreased bone formation. On the other hand, Wase *et al.* (1967), in histologic studies on intact rats, reported enhanced accretion due to thyrocalcitonin administration. Later, Hirsch *et al.* (1969) also obtained indirect evidence that thyrocalcitonin promotes accretion.

When both parathyroid hormone and thyrocalcitonin are given together, their effects on blood calcium tend to cancel out each other. However, the two hormones affect blood phosphate levels in the same manner: both decrease serum phosphate, and this effect is additive when both hormones are given together (Milhaud and Moukhtar, 1966b). In addition, either parathyroid hormone or thyrocalcitonin administration causes phosphaturia (Robinson *et al.*, 1966).

The antagonistic effects of thyrocalcitonin and parathyroid hormone do not appear to be a result of competition for the same acceptor site in bone by the two hormones; thyrocalcitonin is able to cause hypocalcemia in the absence of the parathyroid glands or of parathyroid hormone (Hirsch *et al.*, 1963).

The direct action of thyrocalcitonin on bone was demonstrated first by Aliapoulios *et al.* (1966a), who found that thyrocalcitonin inhibited osteolysis in organ cultures of calvarial bones from young mice. Thyrocalcitonin was very effective in controlling the osteolytic effect of added parathyroid hormone. Other research had indicated that the hypocalcemic effect of thyrocalcitonin was independent of the pituitary gland (Milhaud and Moukhtar, 1965) and of the kidneys and gastrointestinal tract (Munson *et al.*, 1968).

Martin *et al.* (1966) found significant reduction in urinary hydroxyproline excretion after thyrocalcitonin administration. This was further evidence of direct inhibition of bone resorption by thyrocalcitonin, as urinary hydroxyproline -- being derived almost entirely from collagen -- is a good indicator of bone resorption. Foster *et al.* (1966b), at the Royal Postgraduate Medical School, London, observed reduced osteoclast counts and an accumulation of trabecular bone in the tail bones of rats treated chronically with thyrocalcitonin. Young animals apparently are more responsive to thyrocalcitonin than are older animals (Cooper *et al.*, 1967).

Copp, who originated the calcitonin concept, believes that parathyroid hormone is more important than thyrocalcitonin in the control of blood calcium (Copp, 1967). Thyrocalcitonin is believed to be important primarily in the fine adjustment of calcium levels. The two hormones together provide a highly efficient "calciostat" with 2 negative feedback systems. The parathyroids, through secretion of parathyroid hormone, control hypocalcemia; while the ultimobranchial cells, through release of thyrocalcitonin, control hypercalcemia.

Magnesium Deficiency in Rats

Clinical Signs in Magnesium Deficiency. Magnesium has been found to be essential for normal nutrition in all species studied, and a deficiency of the element has profound effects on an animal.

The first known experiment in which magnesium deficiency was produced intentionally in animals was reported by Jehan Leroy (1926) in France. The report of that experiment, in which mice were employed, did not include a description of the clinical signs but indicated only that death resulted from the deficiency. In 1931-1932, reports were published from Johns Hopkins University in which H. D. Kruse, L. R. Orent, and E. V. McCollum described their classic and detailed studies of magnesium deficiency in rats (McCollum and Orent, 1931; Kruse *et al.*, 1932).

The course of dietary magnesium deficiency in rats consists of two fairly distinct phases. The first phase, which usually is evident in less than one week, is manifested by peripheral vasodilation and hyperemia, hyperexcitability, and predisposition to convulsions (Kruse *et al.*, 1932). There are leukocytosis, eosinophilia (Kashiwa and Hungerford, 1958), depletion of tissue mast cell granules (Belanger *et al.*, 1957), and increased histamine in tissue, blood plasma, and urine (Bois *et al.*, 1963; Bois and Beaulnes, 1966). A somewhat later sign is the occurrence of erosive and encrusted skin lesions (Schrader *et al.*, 1937), which eventually may heal even though the rat is in a state of chronic magnesium deficiency.

The second, or chronic, phase is manifested by anorexia, weight loss (or depressed weight gain), whitening and altered shape of the incisor teeth, loss of tone in the feet, gingival hyperplasia, and

subcutaneous edema (Kruse *et al.*, 1932; Klein *et al.*, 1935; Watchorn and McCance, 1937; Schrader *et al.*, 1937).

Lesions in Magnesium Deficiency. During one of the first investigations of experimental magnesium deficiency, Cramer (1932) observed degeneration and calcification in the kidneys of rats fed a low-magnesium diet for several weeks. Cramer's observation has been repeatedly confirmed by others so that, now, calcium salt deposition in the kidneys is the lesion most frequently recognized and associated with chronic magnesium deficiency.

Lowenhaupt *et al.* (1950) presented evidence that the basic lesion in magnesium deficiency was an inflammatory reaction that progressed from minute perivascular accumulations of eosinophilic and neutrophilic polymorphonuclear leukocytes, to necrosis of cells in the same areas. This basic lesion appeared to occur practically throughout the body. More recently, Heggtveit and co-workers in Canada (1964) found that, at least in the heart, the necrotic foci were not necessarily perivascular in distribution and that mononuclear inflammatory cells might predominate over polymorphonuclear cells.

Various workers have reported degenerative lesions in the heart (Greenberg *et al.*, 1936); liver (Schrader *et al.*, 1937); spleen; adrenal gland (Mishra, 1960); skeletal muscle (Lowenhaupt *et al.*, 1950; Heggtveit *et al.*, 1964; Heggtveit, 1965); stomach (Watchorn and McCance, 1937); and brain (Lowenhaupt *et al.*, 1950).

Magnesium deficiency in some instances may predispose to neoplasia. Both thymic tumors (Bois and Beaulnes, 1966) and granulocytic leukemia (Battifora *et al.*, 1968) have been reported to develop in magnesium-deficient rats.

Both the teeth and bones undergo structural changes in chronic magnesium deficiency. Klein *et al.* (1935) described gingival hyperplasia, atrophy of periodontal bone, and striations in the dentin of molar and incisor teeth of rats suggestive of intermittent interference in dentinal calcification. Watchorn and McCance (1937) noticed increased brittleness in the long bones and incisor teeth. The teeth of some rats appeared chalk-white in color. Although no microscopic changes were recognized in the bones, lesions similar to those reported by Klein *et al.* were observed in the teeth.

The dental abnormalities were examined in greater detail by J. I. Irving at the Rowett Research Institute, Aberdeen, Scotland (Irving, 1940). Among other observations, he noted the deposition of calcified material in degenerative ameloblasts and odontoblasts. Irving also mentioned the bands caused by magnesium deficiency in the dentin, and concluded that the bands reflected periodic failure of calcification of the dentinal matrix.

Additional studies of bone changes in magnesium-deficient rats were made by Smith and Nisbet in Scotland (1968). They reported the development of osteoporosis in rats between 3 and 6 weeks after being restricted to a magnesium-deficient diet. No details of the histologic features of the affected bones were given. Yamane and Singer (1953) noted that prolonged magnesium deficiency in hamsters caused the absence of the parallel columns of the metaphyseal bony trabeculae normally seen in the femur. The zone of preliminary calcification just below the epiphyseal line was either atrophic or absent.

Involvement of Magnesium in the Regulation of Calcium

In addition to calcium deposition in the kidneys and skeletal changes, there is other evidence that magnesium deficiency alters calcium metabolism. There is increased concentration or deposition of calcium in skeletal muscle (Watchorn and McCance, 1937) and cardiac muscle (Tufts and Greenberg, 1938). At the same time, an elevation of plasma calcium may be detected in magnesium-deficient rats (MacIntyre and Davidsson, 1958). Also, increasing the levels of dietary calcium or phosphorus aggravates the damage from magnesium deficiency (Tufts and Greenberg, 1938; Colby and Frye, 1951). Cortisone in large doses is synergistic with magnesium deficiency in causing soft tissue calcification in mice (Farnell, 1968).

Gitelman *et al.* (1966, 1968) demonstrated that magnesium metabolism is involved with parathyroid gland activity. They found that removal of the parathyroid glands prevents the development of hypercalcemia in magnesium-deficient rats, as had been reported previously by Heaton and Anderson (1965). Conversely, hypermagnesemia induced a diminution in plasma ionic calcium in the presence of a functional parathyroid gland. It is now apparent, based on the studies of Buckle *et al.* (1968), that the concentration of magnesium in the blood entering the parathyroid gland has an immediate, inverse effect on the amount of parathyroid hormone released by the gland.

In addition to its influence on the release of parathyroid hormone, magnesium is also involved in the activity of the hormone. For example, investigators at the Medical College of Virginia (Estep *et al.*, 1969) have obtained evidence suggesting that, in humans, adequate magnesium levels are required for normal response to parathyroid hormone.

Rasmussen and Tenenhouse of the University of Pennsylvania (1967), on the basis of extensive studies by them and others, have developed a tentative explanation of the biochemical basis of the action of both parathyroid hormone and thyrocalcitonin on bone resorption (osteolysis). Both hormones are believed to act by altering membrane function and thereby ion distributions in cellular and extracellular fluids. Parathyroid hormone is believed to promote the entry of Mg^{++} into bone cells, while thyrocalcitonin promotes the entry of Ca^{++}. Magnesium activates pyrophosphatase, an enzyme involved in osteolysis; by this means parathyroid hormone promotes osteolysis. On the other hand, the entry of Ca^{++} into the cell, caused by thyrocalcitonin, inhibits the pyrophosphatase and osteolysis. There is good evidence that an important intermediate effect of parathyroid hormone is an increase in the levels of adenosine 3′,5′-monophosphate (cyclic AMP) in the target cell (Aurbach *et al.*, 1969). The cyclic AMP is then believed to cause the other cellular changes characteristic of stimulation with parathyroid hormone.

Clinical Importance of Magnesium Deficiency and Thyrocalcitonin

Magnesium Deficiency in Animals. McCandlish (1923) reported the occurrence of convulsions and death in calves restricted to milk diets for abnormally long periods after birth. It was later recognized that the normal amount of magnesium in milk is inadequate to meet the increasing needs of the growing calf (Duncan *et al.*, 1935) and that the signs, which include tetany and death, observed in milk-fed calves were due, at least partially, to dietary magnesium deficiency.

Acute hypomagnesemia as a clinical problem in adult ruminants ("grass tetany", "grass staggers", "wheat pasture poisoning", etc.) has been recognized for many years (Sjollema, 1932). Hypocalcemia may

accompany the hypomagnesemia. The precise cause of grass tetany is unknown, and the disease probably is due to a complex of causative factors. However, it seems that the disease occurs at a time when the physiologic need for magnesium exceeds that which is ingested and absorbed by the animal. This is evident from the fact that supplementation of the diet with magnesium salts will usually prevent the disease (Allcroft and Green, 1938; Allcroft, 1954).

A chronic magnesium deficiency disease has been reported among cattle in the Austrian Alps (Onderscheka *et al.*, 1967). The disease is characterized by loss of condition, lameness, and low blood and tissue magnesium levels. The disease has some similarity to a group of diseases known in various parts of the world as "Manchester wasting disease" (Jamaica); "*enteque seco*" (Argentina); and "*naalehu* disease" (Hawaii) (Arnold and Bras, 1956). In the latter group of diseases, there is extensive soft tissue calcification. Although soft tissue calcification occurs in chronic experimental magnesium deficiency, magnesium deficiency has not been demonstrated to occur in Manchester wasting disease and related diseases.

Magnesium Deficiency in Man. Magnesium deficiency is being recognized as an increasingly important human health problem (Wacker and Parisi, 1968). It occurs in a variety of gastrointestinal and endocrine disorders. It may complicate kwashiorkor (Montgomery, 1960; Caddell and Goddard, 1967; Caddell, 1967). Hyperaldosteronism may be accompanied by clinical magnesium deficiency (Mader and Iseri, 1955). Magnesium deficiency is a cause of seizure disorders of infants. Although hypocalcemia may occur along with the hypomagnesemia in these infants, treatment with magnesium salts is required for the correction of both

the hypomagnesemia and the hypocalcemia (Dooling and Stern, 1967; Paunier *et al.*, 1968).

The nervous signs in alcoholism frequently are attributable to hypomagnesemia (Wacker and Parisi, 1968).

Disorders of Thyrocalcitonin Metabolism in Man. Aliapoulios *et al.* (1966b) found low thyrocalcitonin activity in an extract of a colloid goitrous thyroid gland. Other workers have reported impaired ability in thyroidectomized patients to bring the blood calcium levels back to normal after a test infusion of calcium (Williams *et al.*, 1966). Although such results may indicate a clinical significance of thyrocalcitonin deficiency, the interpretation is complicated by the fact that thyroxin (which might also be deficient in these cases) has an important influence on calcium turnover in bone and on the control of both induced hypocalcemia and hypercalcemia (Jowsey and Detenbeck, 1969).

Thus, there is only meager evidence that thyrocalcitonin deficiency is clinically important. However, there is considerable evidence for the occasional clinical importance of excessive thyrocalcitonin production.

A family affected with osteopetrosis was studied by White and Ahmann (1965). The blood calcium of one member fluctuated from a normal level each morning to a level in the evening sufficiently low to cause seizures. The serum of this patient, especially the evening sample, caused hypocalcemia when injected into rats, suggesting that an excess of thyrocalcitonin in the blood was causing both the osteopetrosis and hypocalcemia.

Mazzuoli *et al.* (1966) reported a case of long-standing diffuse nontoxic goiter characterized by hypocalcemia and tetany. There were hyperplasia of the interfollicular cells and about 14 times the normal concentration of thyrocalcitonin in the thyroid gland, which suggested that excessive endogenous thyrocalcitonin was responsible for the hypocalcemia and tetany.

Chimenes *et al.* (1967) reported the development of thyroid adenomas and hypocalcemia in a woman in which an incomplete thyroidectomy had been done. The hypocalcemia persisted despite treatment with dihydrotachysterol, calcium and parathyroid hormone. A second thyroidectomy caused the calcemia to return to normal. Presumably, the adenomatous thyroid was producing excessive thyrocalcitonin.

Medullary carcinoma of the thyroid gland apparently is a neoplasm of the cells of ultimobranchial origin which produce thyrocalcitonin. Melvin and Tashjian (1968) reported a case of that tumor which was accompanied by hypocalcemia, hypophosphatemia and tetany. There were high levels of thyrocalcitonin in the tumor and in blood plasma, and the condition was referred to as "hyperthyrocalcitonism". Meyer and Abdel-Bari (1968) studied the tissue of a medullary carcinoma by means of electron microscopy, histochemical methods, and bioassay of the tumor for thyrocalcitonin content. The tumor apparently was composed of parafollicular (ultimobranchial) cells, and contained high levels of thyrocalcitonin.

Disorders of Thyrocalcitonin Metabolism in Animals. Rasquin and Rosenbloom (1954) reported hyperplasia of the ultimobranchial glands associated with decalcification of bone and renal calcification in Mexican cave fish (*Astyanax mexicanus*). Although not recognized, there may have

been an unusual demand for secretion of calcitonin by the ultimobranchial glands to compensate for the metabolic imbalance of calcium.

Hereditary osteopetrosis in mice of the "grey-lethal" strain is accompanied by the presence of an unusually large proportion of parafollicular cells in the thyroid glands of affected mice. Osteopetrosis in these mice is believed to be due to overproduction of thyrocalcitonin (Walker, 1966).

Jubb and McEntee (1959) have described hyperplasia and neoplasia of the ultimobranchial cells of the thyroid glands of bovine bulls. The incidence of the tumors was high -- 30% of 129 bulls studied. According to Krook *et al.* (1969), Thomson has recorded a high incidence of vertebral osteophytosis in bulls, which Krook *et al.* believe to be related to overproduction of thyrocalcitonin caused by excessive dietary calcium. They point out that bulls usually are fed amounts of calcium which are based on the physiologic requirements of the lactating cow and which are excessive for the bull.

Capen and Young (1967a) reported that the thyrocalcitonin content of the thyroid gland from cows with parturient paresis was considerably less than in normal postparturient cows. They also observed depletion in number, size, and secretory activity of parafollicular cells of the thyroid glands of diseased cows (Capen and Young, 1967b). They interpreted their findings as indicating that parturient paresis is due to the abrupt release of thyrocalcitonin near the time of parturition, with depression of blood calcium and phosphate caused by the hormone.

Barlet (1968) was able to produce a clinical condition similar to parturient paresis by injecting thyrocalcitonin into lactating Jersey cows, which is consistent with the conclusions of Capen and Young.

In view of the relationship between magnesium and calcium metabolism, thyrocalcitonin may conceivably be involved in the production of grass tetany as well as parturient paresis.

Therapeutic Applications of Thyrocalcitonin. Because of its effect in reducing blood calcium levels and increasing calcium levels in bone, thyrocalcitonin is a logical therapeutic agent for preventing hypercalcemia and soft-tissue calcification (Gabbiani *et al.*, 1968) while promoting mineralization of skeletal tissues.

By administering porcine thyrocalcitonin to 2 patients who had hypercalcemia associated with bony metastases from breast tumors, Foster *et al.* (1966a) were able to obtain useful reduction in the blood calcium levels. Milhaud and Job (1966) successfully treated idiopathic hypercalcemia in an infant with thyrocalcitonin. Bartter (Anon., 1968) used thyrocalcitonin to effect a reduction of the hypercalcemia associated with hyperparathyroidism in a patient with parathyroid gland carcinoma. It has been proposed that thyrocalcitonin may be of value as a prophylactic measure against urinary calculi in humans (Gittes, 1967).

Because of evidence that thyrocalcitonin is most effective where bone turnover is occurring at a rapid rate (Bijvoet *et al.*, 1968), and because bone resorption is accelerated in Paget's disease, thyrocalcitonin has possibilities as a useful agent in treating Paget's disease.

Copp (1969) believes that the most promising therapeutic uses of thyrocalcitonin are in the treatment of nephrocalcinosis and bone wasting occurring in prolonged immobilization, chronic adrenal steroid therapy, and old age.

It has been suggested that synthetic peptides with greater potency than natural thyrocalcitonin may eventually be developed and used in therapy (Webster and Frazer, 1967).

Summary of the Literature Review

Thyrocalcitonin is a peptide hormone produced by the cells originating from the ultimobranchial bodies of the embryo. In mammals, these cells are found in the thyroid gland. The discovery, determination of structure, and laboratory synthesis of thyrocalcitonin were all achieved within a 6-year period starting in 1962.

Thyrocalcitonin causes hypocalcemia and hypophosphatemia while promoting calcification in bone.

Chronic dietary magnesium deficiency causes impaired mineralization of bone and causes deposition of calcium salts in the kidney and other soft tissues. In this respect, the effects of magnesium deficiency are opposite to those of thyrocalcitonin.

Excessive endogenous production of thyrocalcitonin in man or animals has been known to result in hypocalcemia, hypophosphatemia, tetany, coma, and excessive mineralization of bone (e.g., osteopetrosis and spondylosis deformans). Hyperplasia or neoplasia of the ultimobranchial cells is a cause of excessive thyrocalcitonin production. Also, hypersecretion without overgrowth of the secreting cells may occur, as has been suggested in connection with parturient paresis of cattle.

The clinical importance of possible hyposecretion of thyrocalcitonin has not been clearly established.

The metabolism of magnesium and calcium are known to be interrelated, and it is apparent that both magnesium and thyrocalcitonin metabolism can be of considerable importance in human and animal health.

Further research on the relationships between thyrocalcitonin and magnesium and calcium metabolism is therefore desirable. Also, the projected use of thyrocalcitonin as a therapeutic agent makes necessary the determination of any pathologic effects of the administered hormone.

MATERIALS AND METHODS

General

This research consisted of one chronic experiment and one relatively acute experiment.* The experiments were similar in several respects. All animals were weanling male albino rats of the Sprague-Dawley strain.** Throughout both experiments, each rat was housed individually in a suspended, zinc-coated steel cage with front and bottom of wire screen ("hardware cloth").

Clean tap water was available *ad libitum* to each rat from a glass bottle with a black rubber stopper and a stainless steel sip tube. Depending on the experimental group to which it belonged, each rat was fed *ad libitum* with either a magnesium-deficient diet or a control diet identical to the magnesium-deficient diet except for being supplemented with magnesium sulfate. The composition of the diets was essentially that described by Ko *et al.* (1962) and is shown in Table 1. The diets were prepared in the form of 3/8" pellets and supplied by a commercial laboratory.†

A number of rats in each experiment were treated with injections of thyrocalcitonin which had been extracted from hog thyroid glands

---

*The rats were housed while on experiment in Barn 2 of the Veterinary Research Farm, Michigan State University, during the Summer and Fall of 1968. Chemical analyses and the microscopic examination of tissues were performed in Fall, 1968, and in Winter and Spring, 1969.

**Spartan Research Animals, Inc., Haslett, Michigan.

†General Biochemicals Corporation, Chagrin Falls, Ohio.

Table 1. Magnesium-deficient and control diets used in Experiments 1 and 2*

Ingredient	Amount (Gm./Kg., unless otherwise stated)
Casein, Vitamin Free Test	300.00
Dextrose	499.66
Oil, Corn	150.00
Salts (See Below)	21.48
Sodium Bicarbonate [$NaHCO_3$]	12.60
Potassium Bicarbonate [$KHCO_3$]	15.10
Vitamin Mix (See Below)	
Vitamin Mix	
Calcium Pantothenate	0.020
Choline Chloride	1.000
Niacin	0.040
Pyridoxine	0.004
Riboflavin	0.008
Thiamine	0.004
Vitamin A	37,500.000 I.U./Kg.
Vitamin D	5,400.000 I.U./Kg.
Salt Mix	
Calcium Chloride [$CaCl_2$]	5.700
Calcium Phosphate [$CaHPO_4$]	14.300
Cupric Sulfate [$CuSO_4 \cdot 5H_2O$]	0.013
Ferric Citrate [$FeC_6H_5O_7 \cdot 5H_2O$]	1.240
Manganese Sulfate [$MnSO_4 \cdot H_2O$]	0.1739
Sodium Iodide [NaI]	0.030
Zinc Sulfate [$ZnSO_4 \cdot 7H_2O$]	0.024
Magnesium Sulfate [$MgSO_4 \cdot 7H_2O$] (In Control Diet Only)	4.600

*According to Ko *et al.* (1962), the control diet contained the following (in mM/Kg. of diet): Na, 170; K, 152; Ca, 155; P, 113; and Mg, 27. The magnesium-deficient diet contained only 0.7 mM of Mg./Kg.

and purified.* The hormone was in lyophilized form in small vials and, as needed for injection, was dissolved in either a vehicle of 16% gelatin solution (Experiment 1) or in 0.85% sodium chloride solution (Experiment 2). All of the hormone used in this study was from the same production lot, and the potency had been assayed and expressed in MRC units. The MRC unit is an expression of potency which compares the hypocalcemic effect in rats of a given thyrocalcitonin preparation with a standard preparation supplied by the British Medical Research Council. According to Copp (1969), 1.0 MRC unit is equivalent to approximately 4 μg. of pure porcine thyrocalcitonin.

Experiment 1

Experimental Design. Forty rats ranging in weight from 50 to 57 Gm. were randomly divided into 4 groups of 10 each. All the rats in each group were fed either the magnesium-deficient or complete diet, and treated twice every day at 12-hour intervals with either thyrocalcitonin in the gelatin vehicle or with the gelatin vehicle alone.

At any given injection period, all rats were treated with the same amount of the hormone, but the dose given to individual rats varied somewhat, depending on the dilution chosen and the individual body weights. The individual doses of thyrocalcitonin during the experiment varied from 10 to 22 British MRC units/Kg. of body weight per day.

The experimental design is given in Table 2.

Each rat was weighed daily throughout the experiment and examined daily for the presence of lesions or other signs of abnormality. Special attention was given to the occurrence of cutaneous hyperemia,

---

*The thyrocalcitonin was prepared at Armour Pharmaceutical Company, Kankakee, Illinois, and supplied through the courtesy of Dr. J. P. Aldred.

Table 2. Experiment 1. Experimental design and 32-day weight gain

Group No.	No. of Rats*	Diet	Material Injected	Initial Wt. (Gm.)	Final Wt. (Gm.)
1	9	Magnesium-deficient	Thyrocal-citonin	54.6 ± 1.8**	239.0 ± 17.8
2	10	Magnesium-deficient	(Vehicle)†	53.2 ± 2.0	235.4 ± 9.6
3	9	Control	Thyrocal-citonin	53.6 ± 1.7	258.4 ± 12.9
4	9	Control	(Vehicle)	55.1 ± 1.3	283.0 ± 11.5

*All rats were males.

**Values are mean ± standard deviation.

†Vehicle: 16% gelatin solution.

particularly of the pinnae of the ears, and to the occurrence of encrusted erosive lesions of the skin.

Necropsy Procedures. At the end of the diet and treatment period, which lasted 32 days, the rats were anesthetized with ethyl ether and killed by exsanguination. The blood was withdrawn from the abdominal aorta into a hypodermic syringe which had been wet with a film of a solution containing sodium heparin and 0.85% sodium chloride. The plasma was separated by centrifugation and frozen until analyzed for calcium and magnesium.

A gross necropsy examination was performed on each rat. The left rear leg of each rat was removed at the stifle joint without chemical fixation, and the muscle and other soft tissues were carefully removed from the tibia. The foot was left attached to the exposed tibia.

Radiography of Tibias. Radiographs were made of a tibia from each rat for the purpose of comparing bone density, particularly at the newly-developing spongy bone of the metaphysis found adjacent to the proximal epiphysis. In order to minimize individual variation in the quality of the final radiographs, the tibias from all rats were exposed simultaneously on the same film. Because of the possible influence of variations in the angle of impinging X-rays on various parts of the film (thus possibly affecting interpretation of the radiograph), 2 different arrangements of the bones on the film were chosen, by means of a table of random numbers.

The rats' feet, which were left attached to the tibias, were useful in maintaining orientation of the tibias during exposure. Exposures were from a lateral direction.

The film used was "H-D" Class 1, Industrial X-Ray Film, 14" x 17".* Exposure was at 35 kilovolts and 600 milliampere seconds, with no added tube filtration. A nonscreen cassette was used at a focal film distance of 6 feet.

Photography of Tibias. After radiography, the tibias were oven-dried and selected for photography to further demonstrate changes observed in the radiographs.

In order to expose the marrow cavity and metaphyseal spongy bone along a longitudinal plane passing from the medial to lateral side, the anterior portion of each tibia was ground off by means of a wetted, fine-grit waterproof silicon carbide sandpaper. Debris left from the grinding process was removed by vigorous rinsing in a detergent solution, followed by rinsing in distilled water. To further clean the bone of soft tissue, the tibias were then macerated for a period of 6-1/2 to 10 hours each in 1% potassium hydroxide solution and rinsed again in detergent solution and distilled water. Finally, the bones were rinsed successively in absolute alcohol and ethyl ether, and allowed to dry.

Photographs were made with a 35mm. camera** fitted with a close-up lens system.

Chemical Determinations on Blood Plasma. Plasma calcium and magnesium were determined on a Perkin-Elmer Model 303 atomic absorption spectrophotometer. Both calcium and magnesium were determined in the same sample diluted with a solution of lanthanum chloride. The procedures were those recommended by the instrument manufacturer with modifications

---

*General Aniline and Film Corporation.

**Leica M2, on Leitz Reprovit IIa copying stand.

in the dilution procedure as suggested by Young and Booth (1967).

Histologic Techniques. Organs, or portions of organs, from each rat were collected and fixed either in Zenker's solution or in 10% formalin solution containing sodium acetate as a buffer. Tissues containing bones or teeth were decalcified in sodium citrate-formic acid solution. All tissues were infiltrated and embedded in a paraffin-base medium* and sectioned at 6 μ. Sections were stained routinely with hematoxylin and eosin and, in selected cases, special stains were employed to aid in the differentiation of tissue components. The staining procedures were methods in general use (Armed Forces Institute of Pathology, 1960; Lillie, 1965).

Experiment 2

This experiment was a comparison of the hypocalcemic response of magnesium-deficient and nondeficient rats to a single injection of thyrocalcitonin.

Thirty-eight male rats (initial weight range 52 to 61 Gm.) were divided equally into 2 groups which were fed either the magnesium-deficient or the control diet for the duration of the experiment. At the end of a 6-day diet period, the rats in each diet group were divided into 5 subgroups and treated with thyrocalcitonin in 0.85% sodium chloride solution, or with the saline solution alone, as shown in Table 3.

One of the hormone-treated groups fed each diet was pretreated with 2.5 mg./Kg. body weight of magnesium, administered as a single subcutaneous injection of magnesium sulfate solution. The magnesium

---

*"Paraplast", Sherwood Medical Industries, Inc., St. Louis, Mo.

Table 3. Experiment 2. Experimental design and calcium and magnesium in plasma of rats fed diets for 6 days

Diet	No. of Rats	Material Injected* at End of 6-Day Diet Period	Time Lapse Before Blood Withdrawn (hours)	Plasma Calcium (mg./100 ml.)	Plasma Magnesium (mg./100 ml.)
				(mean ± standard deviation)	
Magnesium-deficient	4	Saline	1	11.42 ± 1.08	1.41 ± .84
	3**	TCT	1	7.56 ± .33	1.13 ± .33
	4	TCT	3	9.06 ± 3.83	1.09 ± .33
	4	TCT	5	11.63 ± 1.00	1.09 ± .13
	3	$MgSO_4$ + TCT	1	11.01 ± 2.71	1.34 ± .10
Control	4	Saline	1	9.95 ± .41	2.19 ± .41
	4	TCT	1	7.64 ± 1.04	2.08 ± .32
	4	TCT	3	13.40 ± 4.03	3.01 ± .90
	4	TCT	5	9.94 ± .63	2.06 ± .14
	3	$MgSO_4$ + TCT	1	7.74 ± 1.04	2.23 ± .24

*Material Injected:

$MgSO_4$: Magnesium sulfate heptahydrate in saline, injected subcutaneously 1 hour before TCT. Quantity equivalent to dose of 2.5 mg. of magnesium/Kg. body weight.

Saline: 0.85% sodium chloride in water.

TCT: 5 MRC units of thyrocalcitonin/Kg. body weight, dissolved in saline and injected intraperitoneally.

**One plasma sample in this group was discarded because of an error.

was given in an attempt to restore the blood magnesium to a normal level. These groups were included for the purpose of determining whether any differences in effect of thyrocalcitonin between magnesium-deficient rats and rats on a normal diet were due simply to the difference in blood levels of magnesium, or due to other changes in the rats caused by the magnesium deficiency.

For injection, the thyrocalcitonin was diluted to contain 5 MRC units of the hormone per 1.0 ml. Each rat was weighed and 1.0 microliter (µl.) of the hormone solution administered per Gm. of body weight, by intraperitoneal injection from a 100 µl.-capacity Hamilton microliter syringe. In this way, each rat was treated with 5 MRC units of thyrocalcitonin/Kg. of body weight.

Either 1, 3, or 5 hours after the final injection of thyrocalcitonin or saline solution, each rat was anesthetized with ethyl ether and killed by exsanguination from the abdominal aorta. Calcium and magnesium were determined in heparinized plasma samples by the same procedure as was followed in Experiment 1.

RESULTS

Experiment 1*

Clinical Signs

Hyperexcitability. During the first week of the experiment and thereafter, many of the magnesium-deficient rats would appear hyperexcitable when disturbed. However, extreme excitement or convulsions were observed only rarely. Hyperexcitability was not observed in rats fed the control diet.

No conclusion could be made as to the possible modifying effects of thyrocalcitonin administration on hyperexcitability; there was no clear-cut distinction in the occurrence or degree of hyperexcitability between magnesium-deficient rats treated with thyrocalcitonin and those not treated with the hormone.

Hyperemia. Cutaneous hyperemia, a typical sign of magnesium deficiency in rats, was evaluated primarily by its occurrence in the pinnae of the ears (Figure 1).

Hyperemia of the extremities occurred in all rats fed the magnesium-deficient diet; however, thyrocalcitonin caused a delay in the first

---

*Three of the 40 rats did not survive to the end of the experiment. Rat 2 was injured while escaping during one of the injection periods, and later died. Rats 27 and 40 were removed from the experiment when they developed diarrhea. No data from these 3 rats were included in the evaluation of the results of the experiment.

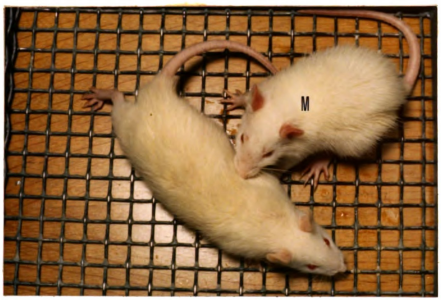

Figure 1. Comparison of magnesium-deficient rat (M) with nondeficient rat. Note the hyperemia (redness) of the ears of the deficient rat--Rat 16, Group 2, Experiment 1.

Figure 2. Comparison of teeth and skin of rats fed a complete diet (left) and magnesium-deficient diet (right) for 32 days. Changes evident in the deficient rat on the right side (Rat 15, Group 2, Experiment 1) of the photograph include chalky-white discoloration of the upper incisor teeth, altered shape of the upper incisors, and 2 encrusted skin lesions (arrows).

appearance of the hyperemia. In the group not treated with thyrocalcitonin, hyperemia first occurred prior to the end of Day 3 of the experiment. In the thyrocalcitonin-treated group, hyperemia did not occur until Day 6.

Skin Lesions. A typical skin lesion of magnesium deficiency is shown (Figure 2). The lesions usually appeared as yellowish crusts covering superficial abrasions or ulcers in the skin. The crusts usually were more extensive than the ulcers which they covered. The lesions were especially prominent about the head, but were observed to occur at various other points over the body.

There was a decrease in severity of skin lesions in rats treated with thyrocalcitonin; and in this group, the lesions healed sooner. For example, on the last day of the experiment, only 2 of 9 of the thyrocalcitonin-treated group had evidence of skin lesions. In contrast, 9 of 10 magnesium-deficient rats not treated with thyrocalcitonin still had skin lesions.

Changes in the Teeth. When the rats had been on the experiment 31 days, the incisor teeth of each rat were examined and grossly visible abnormalities recorded. Definite changes were observed that were due to the magnesium deficiency, but no consistent differences were detected in magnesium-deficient rats between the groups that did or did not receive injections of thyrocalcitonin.

The dental changes are illustrated (Figure 2). A noticeable effect of magnesium deficiency was a change from the normal dull yellow color of the incisor teeth to a pale or chalky-white color. The other obvious change was in the shape of the upper incisors, presumably due to faulty mineralization of the teeth.

Weight Change. The initial and final weights of the rats are given in Table 2. As is usually the case in magnesium deficiency, the deficient rats in general did not gain as rapidly as those fed the control diet. There was a slight retardation of weight gain in the rats treated with thyrocalcitonin, among rats fed the control diet; but this effect of thyrocalcitonin was not observed in the magnesium-deficient groups.

Gross Changes Observed at Necropsy. Changes other than those observed in the living rats were minimal at necropsy. The typical magnesium-deficient rat had a roughened hair coat with occasional skin lesions of the type already described, and less body fat than non-deficient rats. The surface texture of the kidneys of some of the magnesium-deficient rats was uneven, but this change was not prominent.

A portion of liver of Rat 30 was found in the thoracic cavity. This liver tissue was continuous with the rest of the liver in the abdominal cavity by means of a constricted portion that passed through a small, smooth, rounded hernial ring in the right side of the diaphragm. The thymus gland of Rat 35 was cystic. These lesions in Rats 30 and 35 were considered to be incidental and unrelated to the dietary and hormone treatments.

Gingival hyperplasia was evaluated in the lower jaws after they had been fixed in Zenker's fluid and transferred to 80% ethyl alcohol. Detectable gingival hyperplasia occurred only in rats fed the magnesium-deficient diet and was especially prominent next to the molar teeth -- particularly along the buccal side (Figure 3).

In Figure 4, all lower jaws from the magnesium-deficient rats are compared. Hyperplasia was less pronounced in the group of rats treated with thyrocalcitonin.

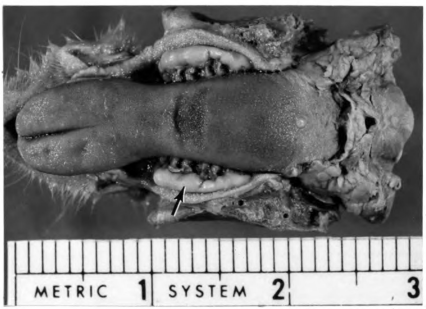

Figure 3. Lower jaw of rat after Zenker's fixation, showing gingival hyperplasia (arrow) caused by magnesium-deficient diet fed for 32 days. Rat 13, Group 2, Experiment 1.

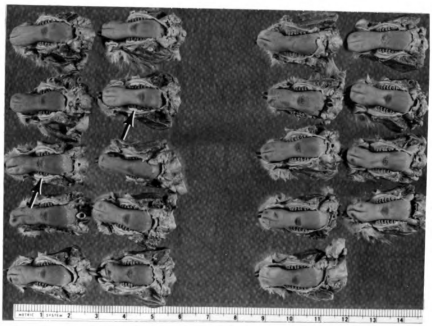

Figure 4. Comparison of lower jaws of rats from Group 1 (right side of photograph) and Group 2 (left side of photograph), Experiment 1. Both groups were fed the magnesium-deficient diet, and Group 1 was in addition treated with thyrocalcitonin. Gingival hyperplasia (arrows) in Group 2 is more pronounced than in Group 1. (Compare this photograph with Figure 3.)

Radiography of the Tibias. The radiographs of the individual tibias were carefully compared. Differences between individual bones appeared to be consistent, regardless of which of the 2 arrangements of bones on the films was being studied. Primary attention was given to the proximal metaphyseal area of each bone.

The most obvious group difference was that the metaphyseal spongy bone in Group 1 (the magnesium-deficient rats treated with thyrocalcitonin) was considerably more dense than in any of the other 3 groups, including the nondeficient rats treated with thyrocalcitonin (Figure 5). Between the 2 groups given the control diet, thyrocalcitonin seemed to be slightly effective in causing some increase in spongy bone density; however, this was a subtle and inconsistent difference.

Between the 2 groups not treated with thyrocalcitonin, magnesium deficiency resulted in the spongy bone of the metaphysis being comparatively abruptly demarcated from the more distal marrow cavity. This demarcation was due apparently to slightly increased density of the spongy bone near the epiphysis, as compared to the other group, and due to less persistence of the bone trabeculae.

Photography of Tibias. Two tibias were photographed, both from rats treated with thyrocalcitonin (Figure 6). Rat 1 was from the magnesium-deficient group, while Rat 25 was from the group fed the control diet. The increased density in the metaphyseal area of the proximal end of the tibia was due to a greater quantity of spongy bone in that area, as can be seen in the photograph.

Histopathologic Changes. Significant changes were noted in the following tissues: heart, kidneys, tibias, gingiva, and teeth. Other tissues, including brain, pituitary gland, thymus, lung, liver, spleen, adrenal

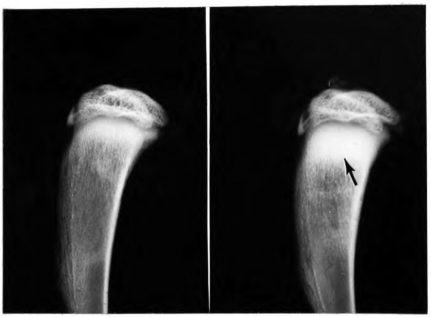

Figure 5. Radiographs of tibias from 2 rats treated with thyrocalcitonin. Rat 23 (left) and Rat 1 (right). Metaphyseal area (arrow) in the magnesium-deficient rat, Rat 1, has greater density because of increased trabecular bone, than in Rat 23, the nondeficient rat.

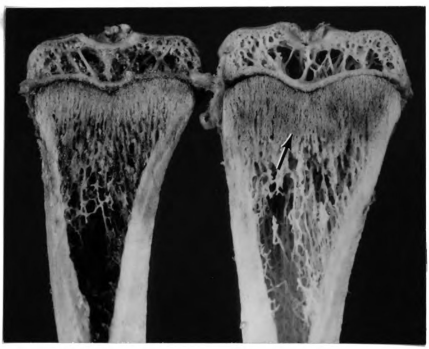

Figure 6. Photograph of rat tibias after bones dried and anterior portion of proximal end of bone removed by sandpaper to expose metaphyseal spongy bone. Rat 25 (left) was fed the control diet. Rat 1 (right) was fed the magnesium-deficient diet. Both rats treated with thyrocalcitonin. Notice the greater quantity of bone (arrow) at the metaphysis of Rat 1.

glands, skeletal muscle, eyeball and adjacent tissues, and skin were examined; but changes in these tissues were minimal or absent in the sections examined.

Heart. Lesions of magnesium deficiency were present in several sections of hearts from magnesium-deficient rats, and the lesions were present in both the thyrocalcitonin-treated and untreated groups. Thyrocalcitonin had no apparent influence on the occurrence of the lesions, which consisted usually of necrosis and replacement of cardiac muscle fibers by accumulations of inflammatory cells. The inflammatory cells were predominantly cells with rounded, moderate-sized nuclei and having basophilic cytoplasm which in some cases formed strands extending to adjacent cells (Figure 7). These cells seemed to be undifferentiated, having prominent nucleoli and showing occasional mitoses. The cells with May-Grunwald-Giemsa stain did not have metachromatic granules in the cytoplasm. The exact histogenesis of these cells was undetermined, but they were likely of a reticuloendothelial type. In addition, the inflammatory exudate contained lymphocytes and polymorphonuclear leukocytes.

Kidneys. There were renal lesions typical of magnesium deficiency in all rats fed the magnesium-deficient diet. These changes included variable tubule cell degeneration and necrosis, and calcium salt deposition. The calcification due to magnesium deficiency, illustrated in Figure 8 and Figure 9, was most prominent at the corticomedullary junction. In the magnesium-deficient rats treated with thyrocalcitonin, the renal lesions generally were less severe, indicating some protective effect of the hormone.

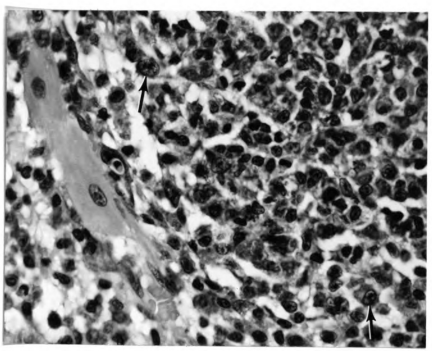

Figure 7. Lesion of magnesium deficiency in the heart after 32 days on diet. Rat 16, Group 2. Cardiac muscle fibers are almost completely replaced by cellular exudate. Mononuclear cells (arrows) predominate in the exudate, and presumably are macrophages or related cells of a reticulo-endothelial origin. H & E stain. x 560.

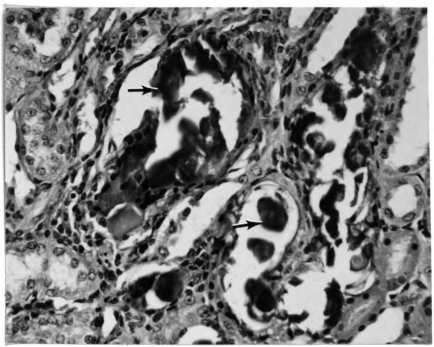

Figure 8. Portion of kidney of magnesium-deficient rat, at corticomedullary junction, showing destruction of tubule epithelium and deposition of calcareous material (arrows) in the lumen of the tubules. Rat 12, fed magnesium-deficient diet for 32 days. H & E stain. x 345.

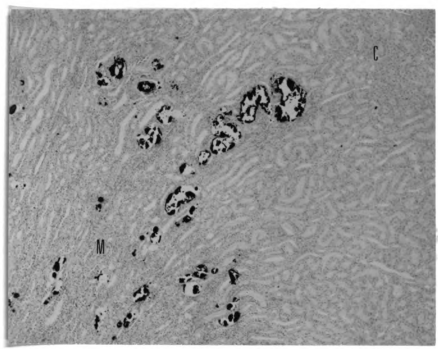

Figure 9. Low-power view of calcium salt deposits (black) in kidney of Rat 12, fed a magnesium-deficient diet for 32 days. Renal cortex (C) and medulla (M). Location and appearance of calcium salt deposits at the corticomedullary junction is a typical feature of magnesium deficiency. Von Kossa stain. x 56.

A second type of renal deposit was observed in rats in this experiment, an example of which is shown in Figure 10. The lesion consisted of laminated deposits of a material that stained a pale blue color with hematoxylin and eosin. The material appeared to form about some unidentified type of nidus located at the basement membranes of the proximal convoluted tubules. Frequently, the layering of material spread in opposite directions from a point at the junction of the basement membranes of adjacent proximal tubules, so that 2 deposits were formed with an appearance reminiscent of an open clam-shell where both halves remained joined by the hinge.

These deposits occurred almost exclusively in thyrocalcitonin-treated rats; however, one deposit of the same type was found in Rat 39, which was fed the control diet and not treated with thyrocalcitonin. Examination of the kidneys of 2 rats not included in the experiment, but of the same age, sex, and breed and fed the control diet under the same conditions, revealed minor deposits of the same type. As these latter 2 rats did not receive any injections, it was apparent that the suspending vehicle used for the hormone was not the cause of the deposition.

The deposits occurred in all of the thyrocalcitonin-treated rats fed the control diet, and the extent of deposition in that group was severe. Many of the deposits became calcified in this group. On the other hand, in the magnesium-deficient group treated with thyrocalcitonin, the deposits were observed in sections of kidney of only 2 of 9 rats, and were of a mild degree in those rats. Thus, the state of magnesium deficiency apparently protected the rats from the deposition of material as promoted by the thyrocalcitonin.

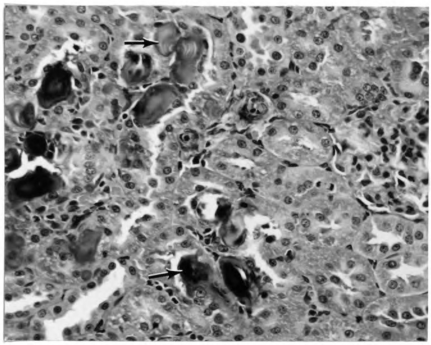

Figure 10. Laminated deposits of amorphous intertubular material (arrows) in cortex of kidney of Rat 30, which was fed the control diet and treated with thyrocalcitonin for 32 days. Some of the material has become mineralized (black appearance). H & E stain. x 345.

The appearance of the deposited material is shown in Figures 10, 11, 12, and 13. In an attempt to identify the material, sections of affected kidneys were subjected to a variety of histochemical stains. The material was PAS-positive, and alcian blue-positive. It gave a staining reaction similar to that of the basement membrane of the glomeruli and tubules, but was shown not to contain reticular fibers when a Laidlaw lithium silver stain was applied. Von Kossa-positive material was observed in many of the more advanced deposits, suggesting that the deposits were being impregnated with calcium salts.

On the basis of the staining reactions, the material was believed to contain mucopolysaccharide and to be similar in nature to the amorphous intercellular substance associated with basement membranes and connective tissue generally (Ham, 1961).

Tibias. Hematoxylin and eosin-stained sections were examined grossly and microscopically. Even without the microscope, the tibias from Group-1 rats (magnesium-deficient diet and thyrocalcitonin treatment) were obviously different from the rest, the metaphyseal spongy bone being much more dense, as was observed in the radiographs. Comparisons of the 2 groups not treated with thyrocalcitonin indicated that the spongy bone in the magnesium-deficient rats was slightly more plentiful near the epiphysis, but the trabeculae had a more nearly parallel arrangement and were somewhat more persistent in the nondeficient rats.

The results of the histologic examination of the tibias confirmed the results of radiography.

Gingiva. The microscopic appearance of the hyperplastic gingiva of magnesium-deficient rats is illustrated (Figure 14). The hyperplasia seemed to be mainly an increase in the connective tissue of the lamina

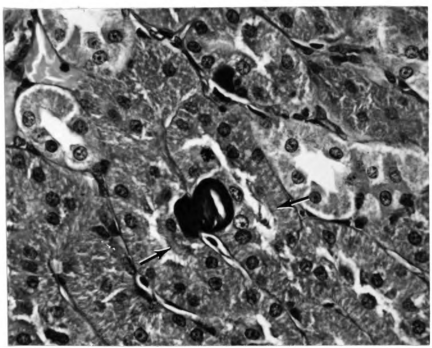

Figure 11. Amorphous deposits (black) of type found almost exclusively in rats treated with thyrocalcitonin. Notice that deposits appear to spread from a point along the basement membrane separating adjacent tubules. The presence of brush borders (arrows) indicates that deposits are between proximal convoluted tubules. Rat 28, control diet and treatment with thyrocalcitonin for 32 days. PAS stain. x 560.

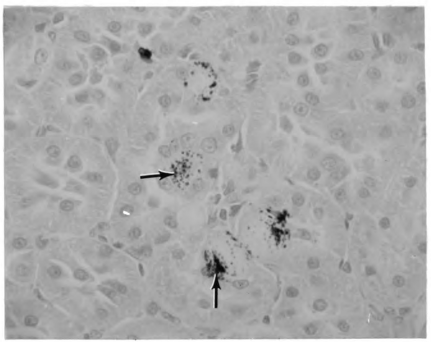

Figure 12. Beginning calcium salt deposition (arrows) in amorphous intercellular material deposited among proximal convoluted tubules in renal cortex. Rat 28, control diet and treatment with thyrocalcitonin for 32 days. Von Kossa stain. x 560.

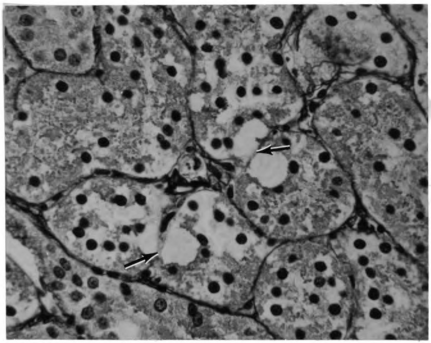

Figure 13. Renal cortex of Rat 28, fed control diet and treated with thyrocalcitonin for 32 days. Special stain for reticular fibers. Reticular fibers appear black. Note lack of staining in the deposits of amorphous material, except for evidence of reticular fibers near points where deposition began (arrows). Laidlaw lithium silver stain. x 560.

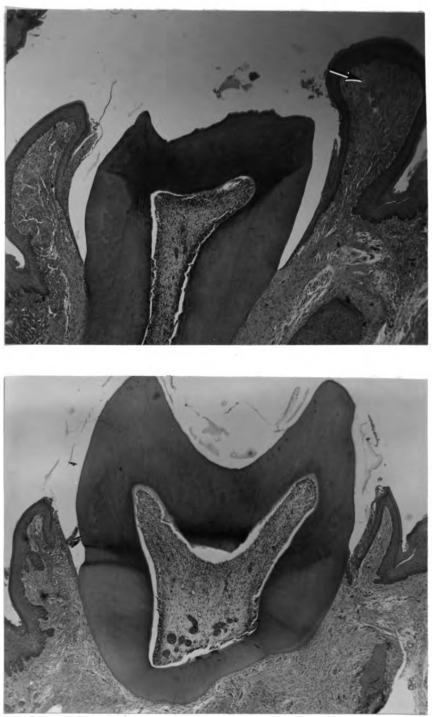

Figure 14. Comparison of gingival hyperplasia in magnesium-deficient rat with gingiva of nondeficient rat, Experiment 1. Sections through 1st lower molar teeth. (A) Rat 16: magnesium-deficient diet for 32 days. (B) Rat 31: complete diet for 32 days. Notice enlargement of gingiva in Rat 16 (arrow) caused by proliferation of the connective tissue of the lamina propria. H & E stain. x 56.

propria. This hyperplasia was less pronounced in the thyrocalcitonin-treated rats.

Teeth. Primary attention was given to the occurrence of changes in the dentin layer of the incisor teeth. Striking changes were observed as a result both of the magnesium deficiency and of treatment with thyrocalcitonin.

The changes due to magnesium deficiency have been described previously (Irving, 1940) and are shown in Figure 15. The dentinal lesions were primarily the presence of bands or stratification in the dentin caused by intermittent interference with the normal calcification process. Multiple bands of this type occurred in the incisor teeth of all magnesium-deficient rats not treated with thyrocalcitonin. In thyrocalcitonin-treated rats, the bands were reduced in number and size, being almost absent in some of the rats. Thyrocalcitonin had a definite protective effect against the dental lesions caused by magnesium deficiency.

In all rats treated with thyrocalcitonin, regardless of diet, the dentin of the incisor teeth was marked with evenly-spaced annular striations (Figures 16 and 17). The average distance between these striations was measured in the teeth of several rats by means of a microscope stage micrometer, and the average distance between individual lines was almost always more than 7 μ but less than 9 μ. Inasmuch as the dentin of the normal rat incisor is laid down at a rate consistently close to 16 μ per day (Schour, 1953), and because the thyrocalcitonin injections were given at 12-hour intervals, each line was believed to be caused by an injection of thyrocalcitonin, which altered in some way the process of dentinal accretion by the odontoblasts.

Figure 15. Cross section through the root of a lower incisor tooth of a rat, at the level of the first molar tooth. Multiple bands (arrows) in dentin caused by magnesium deficiency and consequent intermittent interference with normal process of calcification. Rat 18; fed magnesium-deficient diet for 32 days. H & E stain. x 140.

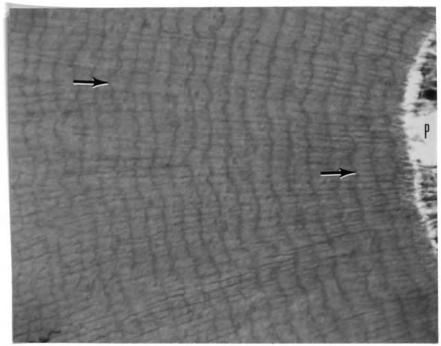

Figure 16. Dentin of incisor tooth of Rat 30. Control diet and twice-daily injections of thyrocalcitonin for 32 days. Lines in dentin (arrows) caused by injections of thyrocalcitonin. A portion of the pulp cavity (P) is visible. H & E stain. x 560.

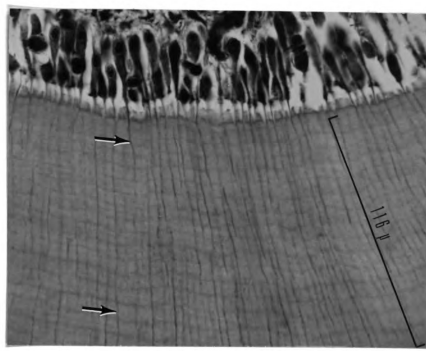

Figure 17. Section through incisor tooth of Rat 8. Magnesium-deficient diet and twice-daily treatment with thyrocalcitonin for 32 days. Pulp cavity (above) and dentin (below) are visible. Notice odontoblastic processes in the dentin (arrows). Fifteen spaces between lines caused by thyrocalcitonin injection are shown within superimposed brackets. The average distance between these dentinal lines is 7.7 μ. Gomori's trichrome stain. x 560.

The incisor dentin of Rat 31, which was fed the control diet and not treated with thyrocalcitonin, is shown in Figure 18. There are no striations due to thyrocalcitonin injection, but faint Leisegang rings about 16 μ apart can be observed. The Leisegang rings reflect the normal daily rhythmic fluctuation in growth rate of the dentin (Schour, 1953).

Similar changes as seen in the dentin of the incisor teeth as a result of either the thyrocalcitonin treatment or magnesium deficiency were observed in longitudinal sections of the 1st molar teeth. However, these changes were less readily evaluated because of the relatively irregular growth pattern of the molar teeth compared to the incisors.

Blood Plasma Calcium and Magnesium. The plasma calcium and magnesium levels at the end of the experimental period are summarized in Table 4. These data were subjected to an analysis of variance (Steel and Torrie, 1960) as a means of estimating the significance of group comparisons.

Although the mean plasma calcium levels in the rats fed the magnesium-deficient diet were numerically slightly higher than in rats given the control diet, this difference was statistically insignificant. There was no significant effect on plasma calcium by either the thyrocalcitonin treatment or magnesium deficiency.

Magnesium deficiency caused a highly significant reduction in plasma magnesium, as would be expected. In the experiment taken as a whole, thyrocalcitonin had a highly significant effect in elevating plasma magnesium. When individual groups were compared, the effect of thyrocalcitonin in elevating plasma magnesium was not significant in the rats fed the complete diet; but thyrocalcitonin treatment caused a significant elevation of plasma magnesium in the magnesium-deficient rats.

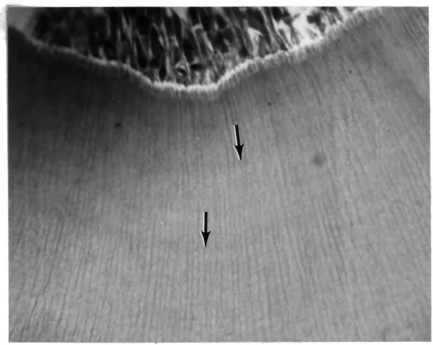

Figure 18. Section through incisor tooth of Rat 31. Complete diet and no other treatment. Compare with Figures 16 and 17. No lines due to thyrocalcitonin injection; but normal, faint Leisegang lines (arrows) are present. H & E stain. x 560.

Table 4. Experiment 1. Blood plasma calcium and magnesium levels after 32 days

Group No.	No. of Rats	Diet	Material Injected	Plasma Ca (mg./100 ml.)	Plasma Mg (mg./100 ml.)
1	8	Magnesium-deficient	Thyrocalcitonin	11.17 ± .56*	.66 ± .19*
2	10	Magnesium-deficient	(Vehicle)**	11.22 ± 1.32	.49 ± .08
3	9	Complete	Thyrocalcitonin	10.98 ± .70	2.03 ± .30
4	9	Complete	(Vehicle)**	10.53 ± .67	1.86 ± .10

*Values are mean ± standard deviation.

**16% gelatin solution.

Experiment 2

This was an acute type of experiment, and histopathologic studies were not appropriate. Blood plasma calcium and magnesium levels were determined and are summarized in Table 3.

By comparison with the other groups, it is apparent that the injection of magnesium sulfate had only a transient if any perceptible effect in increasing the blood magnesium. The cause of the relatively low plasma calcium in the control versus magnesium-deficient rats pretreated with magnesium sulfate (7.74 $\pm$ 1.04, vs. 11.01 $\pm$ 2.71 mg./100 ml.) is not known.

There were no significant differences in plasma magnesium among the various groups, except that the rats fed the magnesium-deficient diet had lower plasma magnesium than those fed the complete diet.

The plasma calcium of the 8 groups not pretreated with magnesium sulfate are plotted in Figure 19. The values from the saline-treated group were taken as the zero-time values. The principal finding of Experiment 2, as illustrated in Figure 19, was a prolongation of the hypocalcemic effect of thyrocalcitonin in the magnesium-deficient rats. At 1 hour after injection of thyrocalcitonin, the plasma calcium levels were about equal in the control and deficient rats but the levels were below the zero-time values. Three hours after injection, the plasma calcium in the magnesium-deficient group was still below the pretreatment value, while the plasma calcium of the nondeficient group had returned to and seemed to exceed the pretreatment value. Five hours after thyrocalcitonin injection, plasma calcium values in both diet groups were at the pretreatment levels.

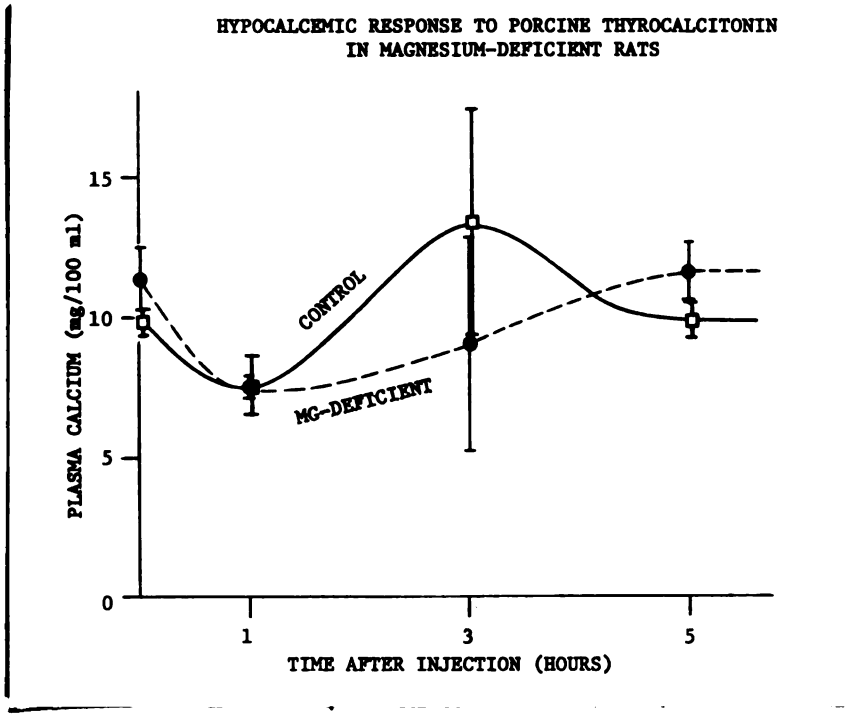

Figure 19. Hypocalcemic response of rats in Experiment 2 to a single injection of thyrocalcitonin. The hypocalcemic effect of the thyrocalcitonin is prolonged in rats fed the magnesium-deficient diet for 6 days before treatment with the hormone, as compared to rats fed the control diet for the same period of time.

DISCUSSION

It is apparent from the data that there were certain significant interrelationships between the effects in rats of dietary magnesium deficiency and exogenous thyrocalcitonin.

Clinical Signs

The observed beneficial effect of thyrocalcitonin in Experiment 1 in delaying the occurrence of cutaneous hyperemia and in lessening the severity of the skin lesions and gingival hyperplasia may have been associated with the observed elevation of plasma magnesium in the magnesium-deficient rats treated with the hormone. The hormone apparently affected the distribution of magnesium in such a way that the effects of the magnesium deficiency did not have their full detrimental impact in peripheral areas of the body.

In contrast to the result in Experiment 1, there was no observable effect of thyrocalcitonin in Experiment 2 in increasing plasma magnesium levels. However, this difference conceivably could be attributable to the difference in the time lapse from hormone injection to blood withdrawal between the two experiments. In Experiment 2, blood samples were withdrawn regularly at either 1, 3, or 5 hours after thyrocalcitonin injection. In Experiment 1, the time lapse between the last injection of thyrocalcitonin and blood withdrawal varied from 4 hours, 57 minutes, to 11 hours, 35 minutes, and was in excess of 5 hours in all but one rat. Therefore, the observed plasma levels of magnesium in Experiments

1 and 2 would not necessarily be expected to be comparable. It may have been that the plasma magnesium levels during most of a 24-hour period were elevated by the thyrocalcitonin, but that this effect did not occur until several hours after injection of the hormone.

Also, it should be recognized that the effects of the hormone after a 6-day diet period may have been somewhat different than after a 32-day diet period -- at which time the extent of hypomagnesemia was far greater.

It is of interest that cortisone treatment has been reported to delay the occurrence of hyperemia in magnesium-deficient rats (Farnell, 1966). The precise modes of action of cortisone and thyrocalcitonin in delaying hyperemia are unknown.

Lesions of Magnesium Deficiency in the Kidney

The beneficial effect of thyrocalcitonin in reducing the severity of calcium salt deposition in the kidney of magnesium-deficient rats probably is related to the observed accompanying increase in quantity of spongy bone in the tibia. Presumably, the thyrocalcitonin caused a relative shift of calcium from the kidney, and possibly other soft tissues, to skeletal tissues.

The protective effect of thyrocalcitonin against the renal calcification of magnesium deficiency is similar to the protective effect of thyrocalcitonin that has been reported in other types of experimental soft tissue calcification (Gabbiani *et al.*, 1968; Cote *et al.*, 1968).

Bone Changes

The marked increase in metaphyseal spongy bone in the tibias of magnesium-deficient rats treated with thyrocalcitonin, in comparison with thyrocalcitonin-treated rats fed a complete diet, is not readily explained.

The results of Experiment 2 -- in which the hypocalcemic effect of thyrocalcitonin was found to be prolonged in magnesium-deficient rats -- support a conclusion that the increase in bone density in thyrocalcitonin-treated, magnesium-deficient rats was attributable to a prolonged effect of the hormone. If this conclusion is correct, the prolonged effect might be explained on the basis of reduced degradation or excretion of the hormone.

Because all these rats had normal parathyroid glands, a possible role of parathyroid hormone must be considered.

Heaton and Anderson (1965), who used parathyroidectomized rats, reported that magnesium deficiency did not cause the usual hypercalcemia and nephrocalcinosis seen in intact, magnesium-deficient rats. This was interpreted as an indication that induced hyperparathyroidism was the cause of the hypercalcemia and the nephrocalcinosis usually seen. Such an interpretation is consistent with the finding of an inverse effect of plasma magnesium levels on the secretion rate of parathyroid hormone (Gitelman *et al.*, 1966). Assuming that the magnesium-deficient rats in Experiment 1 were hyperparathyroid, and other factors being equal, one would expect reduced density of the metaphyseal spongy bone in the deficient rats as compared to the non-deficient rats, even though both groups were treated with the hormone. It does not seem that hyperparathyroidism was a significant influence on the bone morphology, if hyperparathyroidism actually occurred.

There is evidence that magnesium ions are necessary for the functioning of parathyroid hormone in the bone cells (Rasmussen and Tenenhouse, 1967), which invites an interpretation that the increased bone density was attributable to a decrease in parathyroid hormone action at the cellular level. The slight increase in metaphyseal density in tibias of magnesium-deficient rats not treated with thyrocalcitonin is consistent with such an explanation. In addition, there is also evidence that magnesium ions inhibit calcium uptake *in vitro* in collagen fibers (Wadkins, 1968).

Intertubular Deposits in the Renal Cortex

Although the condition of magnesium deficiency was synergistic with thyrocalcitonin administration in increasing metaphyseal spongy bone in the tibias, magnesium deficiency inhibited the deposition, as caused by thyrocalcitonin, of material in the renal cortex that resembled in some respects the amorphous intercellular substance of connective tissue.

It seems possible that thyrocalcitonin caused an intercellular substance in the kidney to be deposited by the cells of the convoluted tubules in a way analogous to that in which organic matrix is laid down by osteoblasts and odontoblasts. The eventual occurrence of calcification of the deposited material is consistent with such a supposition.

Changes in the Teeth

The protective effect of thyrocalcitonin against the dentinal lesions in the incisor teeth of magnesium-deficient rats is consistent with the increase in spongy bone that occurred in deficient rats treated with the hormone. Although a protective effect of thyrocalcitonin against the teeth lesions was observed microscopically, the protective

effect was insufficient to be observed grossly on the day before the rats were killed.

The occurrence of lines in the dentin of incisor teeth in response to thyrocalcitonin administration is regarded as one of the more significant results of Experiment 1. These lines were a precise, apparently permanent record of each injection of thyrocalcitonin. Presumably, thyrocalcitonin could be used to mark teeth chronologically for various research purposes. The lines presumably were indicative of a change in the dentinal calcification rate following each injection of thyrocalcitonin, but an effect on collagen formation also is possible.

General

The manifestations of magnesium deficiency that were inhibited by thyrocalcitonin administration included cutaneous hyperemia, discrete skin lesions, gingival hyperplasia, renal calcification, dentinal lesions in incisor teeth, and hypomagnesemia. Therefore, a definite protective effect of the hormone in magnesium-deficient rats was demonstrated.

While these results indicate a significant interrelationship between the metabolism of magnesium, calcium, and thyrocalcitonin, the potential clinical importance of the results is a matter which can only be speculated upon at this time. Although thyrocalcitonin may offer protection against magnesium deficiency, magnesium salt administration in magnesium deficiency would seem to be a more direct approach to therapy than the administration of thyrocalcitonin. However, thyrocalcitonin may possibly be a useful therapeutic agent in other conditions where there is an abnormal shift of calcium salts from skeletal tissues to soft tissues, as was discussed in the literature review.

The enhanced effect of thyrocalcitonin in magnesium deficiency, in promoting trabecular bone formation in the tibia, suggests that such enhancement of thyrocalcitonin activity in clinical magnesium deficiency may be a factor in the occurrence of the hypocalcemia that may accompany hypomagnesemia. This might explain the reported requirement for treatment with magnesium salts in order to obtain any lasting increase in the blood calcium in some infants with both hypomagnesemia and hypocalcemia (Dooling and Stern, 1967; Paunier *et al.*, 1968).

The effect of thyrocalcitonin in causing deposition in the kidneys of intercellular material that eventually became calcified demonstrates that caution should be exercised in the prolonged use of thyrocalcitonin as a therapeutic agent. The protective effect of magnesium deficiency in preventing the renal deposits caused by thyrocalcitonin indicates that the magnesium nutritional status of the patient may be an important consideration where thyrocalcitonin therapy is contemplated.

The demonstrated effect of thyrocalcitonin on the laying down of dentin in the teeth of magnesium-deficient and nondeficient rats indicates that thyrocalcitonin may be an important factor in dental physiology and health.

Further research suggested by the present results include investigations of the toxicology of thyrocalcitonin with and without variations in the dietary intake of magnesium, and of other substances such as protein and calcium which are known to influence the magnesium dietary requirement. Research is suggested to more clearly define the influence of thyrocalcitonin and magnesium on the development of teeth and bones.

In view of the frequent occurrence of atherosclerosis and mineral deposition in arteries in patients with hypothyroidism, a possibility exists of altered thyrocalcitonin metabolism as a contributory factor

in atherosclerosis. The possible influence of impaired thyrocalcitonin metabolism in blood vascular disease should be studied.

Because of the physical separation of the ultimobranchial and thyroid tissue in birds (allowing selective extirpation of the ultimobranchial tissue while leaving the thyroid tissue intact), along with the unusual importance of calcium metabolism in laying birds, the role of calcitonin in species such as chickens, ducks, etc., should be thoroughly studied. In particular, the effect of ultimobranchialectomy should be studied in birds of various ages, fed diets varying in magnesium and calcium content.

A comparison of the modes of action of thyrocalcitonin and cortisone, both of which inhibit the cutaneous hyperemia of magnesium deficiency in rats, would be of interest, particularly since these 2 substances have opposite effects on mineral metabolism when given chronically.

The role of thyrocalcitonin in grass tetany of ruminants is a subject which should be studied. This is especially true because of the occurrence of hypomagnesemia in grass tetany plus the demonstrated relationship between thyrocalcitonin and magnesium metabolism. Similar studies are warranted on hypomagnesemia in man.

The role of magnesium metabolism in parturient paresis of cattle should be thoroughly investigated, in view of the relationships between the physiology of magnesium, thyrocalcitonin, and parathyroid hormone -- plus the apparent involvement of thyrocalcitonin in the occurrence of parturient paresis.

SUMMARY

The interrelationships between magnesium, calcium, and the hypocalcemic hormone, thyrocalcitonin, were investigated in 2 experiments using rats. In the first experiment, 40 rats were divided into 2 groups and fed either a magnesium-deficient or control diet for 32 days. During the diet period, one-half of the rats fed each diet were given twice-daily injections of thyrocalcitonin (porcine origin). The experiment lasted 32 days, at which time the rats were killed and blood and tissues saved for chemical and histopathologic study. In a second experiment, 38 rats were divided into 2 groups and fed the magnesium-deficient or control diet. At the end of a 6-day period, rats from each group were given single injections of thyrocalcitonin and samples of blood collected at 1, 3, or 5 hours after injection.

The principal results were as follows:

1. Thyrocalcitonin caused a delay in the occurrence of cutaneous hyperemia in magnesium-deficient rats. The skin lesions and gingival hyperplasia of magnesium deficiency were less severe in thyrocalcitonin-treated rats.

2. The effect of thyrocalcitonin in increasing the density of metaphyseal spongy bone was enhanced in magnesium-deficient rats, as determined radiographically, by gross examination of the opened bone, and by histologic examination.

3. Thyrocalcitonin had a protective effect against the renal calcification caused by magnesium deficiency.

4. Thyrocalcitonin promoted the deposition in the renal cortex of a material resembling the amorphous intercellular substance of connective tissues. This deposition was inhibited by magnesium deficiency.

5. Thyrocalcitonin had a protective effect against the microscopic dentinal lesions in the teeth of magnesium-deficient rats.

6. Thyrocalcitonin administration consistently caused the occurrence of regularly-spaced concentric lines in the dentin of the teeth, each line corresponding to a single injection of the hormone. The lines were due probably to an altered rate of dentin deposition or calcification.

7. Thyrocalcitonin caused an elevation of plasma magnesium in the first experiment. In the second experiment, the hypocalcemic effect of the thyrocalcitonin was prolonged in magnesium-deficient rats, as compared to the effect of the hormone in nondeficient rats.

The results indicated a significant interrelation in effect of exogenous thyrocalcitonin and metabolism of magnesium and calcium.

BIBLIOGRAPHY

Aliapoulios, M. A., Goldhaber, P., and Munson, P. L.: Thyrocalcitonin Inhibition of Bone Resorption Induced by Parathyroid Hormone in Tissue Culture. Science, 151, (1966a): 330-331.

Aliapoulios, M. A., Voelkel, E. F., and Munson, P. L.: Assay of Human Thyroid Glands for Thyrocalcitonin Activity. J. Clin. End. and Met., 26, (1966b): 897-901.

Allcroft, W. M., and Green, H. H.: Seasonal Hypomagnesemia of the Bovine, Without Clinical Symptoms. J. Comp. Path. and Ther., 51, (1938): 176-191.

Allcroft, Ruth: Hypomagnesemia in Cattle. Vet. Rec., 66, (1954): 517-522.

Anonymous: Key Thyrocalcitonin Puzzles Solved. J.A.M.A., 204(II), (1968): 33.

Arey, L. B.: *Developmental Anatomy. A Textbook and Laboratory Manual of Embryology.* 5th ed. W. B. Saunders, Philadelphia, Pa., 1946.

Armed Forces Insitute of Pathology: *Manual of Histologic and Special Staining Technics.* 2nd ed. McGraw-Hill, New York, 1960.

Arnold, R. M., and Bras, G.: Observations on the Morbid Anatomy and Histology of Manchester Wasting Disease of Cattle in Jamaica and Related Conditions in Other Countries of the Americas. Am. J. Vet. Res., 17, (1956): 630-639.

Aurbach, G. D., Potts, J. T., Jr., Chase, L. R., and Melson, G. L.: Polypeptide Hormones and Calcium Metabolism. Ann. Int. Med., 70, (1969): 1243-1265.

Barlet, J-P.: *Induction expérimentale d'un syndrome analogue à la fièvre vitulaire par administration de thyrocalcitonine à des vaches en cours de lactation.* C. R. Acad. Sci., Paris, Ser. D, 267, (1968): 2010-2013.

Battifora, H. A., McCreary, P. A., Hahnemann, B. M., Laing, G. H., and Hass, G. M.: Chronic Magnesium Deficiency in the Rat. Studies of Chronic Myelogenous Leukemia. Arch. Path., 86, (1968): 610-620.

Baylink, D., Morey, E., and Rich, C.: Effect of Calcitonin on the Rates of Bone Formation and Resorption in the Rat. Endocrinol., 84, (1969): 261-269.

Belanger, L. F., Van Erkel, G. A., and Jakerow, A.: Behavior of the Dermal Mast Cells in Magnesium-Deficient Rats. Science, 126, (1957): 29-30.

Bijvoet, O. L. M., van der Sluys Veer, J., and Jansen, A. P.: Effects of Calcitonin on Patients with Paget's Disease, Thyrotoxicosis, or Hypercalcemia. Lancet, I, (1968): 876-881.

Bois, P., Gascon, A., and Beaulnes, A.: Histamine-Liberating Effect of Magnesium Deficiency in the Rat. Nature, 197, (1963): 501-502.

Bois, P., and Beaulnes, A.: Histamine, Magnesium Deficiency, and Thymic Tumors in Rats. Canad. J. Physiol. & Pharm., 44, (1966): 373-377.

Buckle, R. M., Care, A. D., Cooper, C. W., and Gitelman, H. J.: The Influence of Plasma Magnesium Concentration on Parathyroid Hormone Secretion. J. Endocrinol., 42, (1968): 529-534.

Bussolati, C., and Pearse, A. G. E.: Immunofluorescent Localization of Calcitonin in the "C" Cells of Pig and Dog Thyroid. J. Endocrinol., 37, (1967): 205-210.

Caddell, J. L., and Goddard, D. R.: Studies in Protein-Calorie Malnutrition. I. Chemical Evidence for Magnesium Deficiency. New Eng. J. Med., 276, (1967): 533-535.

Caddell, J. L.: Studies in Protein-Calorie Malnutrition. II. A Double-Blind Clinical Trial to Assess Magnesium Therapy. New Eng. J. Med., 276, (1967): 535-540.

Capen, C. C., and Young, D. M.: Thyrocalcitonin: Evidence for Release in a Spontaneous Hypocalcemic Disorder. Science, 157, (1967a): 205-206.

Capen, C. C., and Young, D. M.: The Ultrastructure of the Parathyroid Glands and Thyroid Parafollicular Cells of Cows with Parturient Paresis and Hypocalcemia. Lab. Invest., 17, (1967b): 717-737.

Chimenes, H., Klotz, H. P., and Mercadier, M.: Post-Operatory Pseudo-adenomatous Relapse of a Patient with the Thyrotoxic Form of Hashimoto's Disease. Repair by Reintervention of a Major Hypocalcemia: Sequelae of the First Operation and Probable Role of Thyrocalcitonin. Ann. Endocrinol., 28, (1967): 507-513.

Colby, R. W., and Frye, C. M.: Effect of Feeding Various Levels of Calcium, Potassium, and Magnesium to Rats. Am. J. Physiol., 166, (1951): 209-212.

Cooper, C. W., Hirsch, P. F., Toverud, S. U., and Munson, P. L.: An Improved Method for the Biological Assay of Thyrocalcitonin. Endocrinol., 81, (1967): 610-616.

Copp, D. H., Cameron, E. C., Cheney, B., Davidson, A. G. F., and Henze, K. G.: Evidence for Calcitonin--A New Hormone from the Parathyroid That Lowers Blood Calcium. Endocrinol., 70, (1962): 638-649.

Copp, D. H.: Hormonal Control of Hypercalcemia. Historic Development of the Calcitonin Concept. Am. J. Med., 43, (1967): 648-655.

Copp, D. H., Lockcroft, D. W., and Kueh, Y.: Ultimobranchial Origin of Calcitonin. Hypocalcemic Effect of Extracts from Chicken Glands. Canad. J. Physiol. and Pharm., 45, (1967): 1095-1099.

Copp, D. H.: Calcitonin and Parathyroid Hormone. Ann. Rev. of Pharmacology, 9, (1969): 327-344.

Cote, G., Gabbiani, G., Tuchweber, B., and Deziel, C.: Effect of Thyrocalcitonin and Thyroxine on Experimental Metastatic Calcification. Acta Endocrinol., 59, (1968): 362-370.

Cramer, W.: Experimental Production of Kidney Lesions by Diet. Lancet, 223, (1932): 174-175.

Dooling, E. C., and Stern, L.: Hypomagnesemia with Convulsions in a Newborn Infant. Canad. Med. Assn. J., 97, (1967): 827-831.

Duncan, C. W., Huffman, C. F., and Robinson, C. S.: Magnesium Studies in Calves. I. Tetany Produced by a Ration of Milk or Milk with Various Supplements. J. Biol. Chem., 108, (1935): 35-44.

Estep, H., Shaw, W. A., Watlington, C., Hobe, R., Holland, W., and Tucker, St.G.: Hypocalcemia due to Hypomagnesemia and Reversible Parathyroid Hormone Unresponsiveness. J. Clin. Endocrinol. and Metabol., 29, (1969): 842-848.

Farnell, D. R.: Therapeutic Effect of Cortisone in Magnesium Deficient Rats. J. Ala. Acad. Sci., 36, (1965): 194.

Farnell, D. R.: Cortisone Treatment in Magnesium Deficiency of Rats. Am. J. Vet. Res., 27, (1966a): 415-418.

Farnell, D. R.: Magnesium Deficiency and Cortisone Treatment in Mice. J. Ala. Acad. Sci., 37, (1966b): 279.

Farnell, D. R.: Functional and Structural Effects of Magnesium Deficiency and Cortisone Treatment in Mice. Am. J. Vet. Res., 29, (1968): 1695-1706.

Foster, G. V., Joplin, G. F., MacIntyre, I., and Melvin, K. E. W.: Effect of Thyrocalcitonin in Man. Lancet, I, (1966a): 107-109.

Foster, G. V., Doyle, F. H., Bordier, P., and Matrajt, H.: Effect of Thyrocalcitonin on Bone. Lancet, II, (1966b): 1428-1431.

Gabbiani, G., Tuchweber, B., Coté, G., and Lefort, P.: Action of Thyroxine and Calcitonin on Experimental Soft-Tissue Calcification. Calc. Tiss. Res., 2, (1968): 30-37.

Gitelman, H. J., Kukol, S., and Welt, L. G.: Inhibition of Parathyroid Gland Function by Hypermagnesemia. Fed. Proc., 25, (1966): 495.

Gitelman, H. J., Kukolj, S., and Welt, L. G.: The Influence of the Parathyroid Glands on the Hypercalcemia of Experimental Magnesium Depletion in the Rat. J. Clin. Invest., 47, (1968): 118-126.

Gittes, R. F.: Thyrocalcitonin: A New Look at Calcium Homeostasis. Urol. Int., 22, (1967): 285-300.

Greenberg, D. M., Anderson, C. E., and Tufts, E. V.: Pathological Changes in the Tissues of Rats Reared on Diets Low in Magnesium. J. Biol. Chem., 114, (1936): xliii.

Ham, A. W.: *Histology*. 4th ed. J. B. Lippincott, Philadelphia, Pa., 1961.

Heaton, F. W., and Anderson, C. K.: The Mechanisms of Renal Calcification Induced by Magnesium Deficiency in the Rat. Clin. Sci., 28, (1965): 99-106.

Heggtveit, H. A., Herman, L., and Mishra, R. K.: Cardiac Necrosis and Calcification in Experimental Magnesium Deficiency. A Light and Electron Microscope Study. Am. J. Path., 45, (1964): 757-782.

Heggtveit, H. A.: Myopathy in Magnesium Deficiency. Life Sciences, 4, (1965): 69-72.

Hirsch, P. F., Gauthier, G. F., and Munson, P. L.: Thyroid Hypocalcemic Principle and Recurrent Laryngeal Nerve Injury as Factors Affecting the Response to Parathyroidectomy in Rats. Endocrinol., 73, (1963): 244-252.

Hirsch, P. F., Orimo, H., and Sliwowski, A.: Evidence that Thyrocalcitonin (TC) Increases Bone Accretion in Rats. Fed. Proc., 28, (1969): 383.

Irving, J. T.: The Influence of Diets Low in Magnesium upon the Histological Appearance of the Incisor Tooth of the Rat. J. Physiol., 99, (1940): 8-17.

Jowsey, J., and Detenbeck, L. C.: Importance of Thyroid Hormones in Bone Metabolism and Calcium Homeostasis. Endocrinol., 85, (1969): 87-95.

Jubb, K. V., and McEntee, K.: The Relationship of Ultimobranchial Remnants and Derivatives to Tumors of the Thyroid Gland in Cattle. Cornell Vet., 49, (1959): 41-69.

Kashiwa, H. K., and Hungerford, G. F.: Blood Leucocyte Response in Rats Fed a Magnesium-Deficient Diet. Proc. Soc. Exp. Biol. Med., 99, (1958): 441-443.

Klein, H., Orent, E. R., and McCollum, E. V.: The Effects of Magnesium Deficiency on the Teeth and Their Supporting Structures in Rats. Am. J. Physiol., 112, (1935): 256-262.

Ko, K. W., Fellers, F. X., and Craig, J. M.: Observations on Magnesium Deficiency in the Rat. Lab. Invest., 11, (1962): 294-305.

Kraintz, L., and Puil, E. A.: Absence of Hypocalcemic Activity in Chicken Thyroid. Canad. J. Physiol. and Pharm., 45, (1967): 1099-1103.

Krook, L., Lutwak, L., and McEntee, K.: Dietary Calcium, Ultimobranchial Tumors and Osteopetrosis in the Bull. Syndrome of Calcitonin Excess? Am. J. Clin. Nutr., 22, (1969): 115-118.

Kruse, H. D., Orent, E. R., and McCollum, E. V.: Studies on Magnesium Deficiency in Animals. I. Symptomatology Resulting from Magnesium Deprivation. J. Biol. Chem., 96, (1932): 519-539.

Leroy, J.: *Nécessité du magnésium pour la croissance de la Souris.* Compt. rend. Soc. biol., 94, (1926): 431-433.

Lillie, R. D.: *Histopathologic Technic and Practical Histochemistry.* 3rd ed. McGraw-Hill, New York, 1965.

Lowenhaupt, E., Schulman, M. P., and Greenberg, D. M.: Basic Histologic Lesions of Magnesium Deficiency in the Rat. Archives of Path., 49, (1950): 427-433.

MacIntyre, I., and Davidsson, D.: The Production of Secondary Potassium Depletion, Sodium Retention, Nephrocalcinosis and Hypercalcaemia by Magnesium Deficiency. Biochem. J., 70, (1958): 456-462.

Mader, I. J., and Iseri, L. T.: Spontaneous Hypopotassemia, Hypomagnesemia, Alkalosis and Tetany due to Hypersecretion of Corticosterone-Like Mineralocorticoid. Am. J. Med., 19, (1955): 976-988.

Martin, T. J., Robinson, C. J., and MacIntyre, I.: The Mode of Action of Thyrocalcitonin. Lancet, I, (1966): 900-902.

Mazzuoli, G. F., Coen, G., and Baschieri, L.: Thyrocalcitonin Excess Syndrome. Lancet, II, (1966): 1192-1193.

McCandlish, A. C.: Studies in the Growth and Nutrition of Dairy Calves. V. Milk as the Sole Ration for Calves. J. Dairy Sci., 6, (1923): 54-66.

McCollum, E. V., and Orent, E. R.: Effects on the Rat of Deprivation of Magnesium. J. Biol. Chem., 92, Scientific Proceedings, (1931): XXX.

McLean, F. C., and Urist, M. R.: *Bone.* The Univ. of Chicago Press, Chicago, Ill., 1955.

Melvin, K. E. W., and Tashjian, A. H., Jr.: The Syndrome of Excessive Thyrocalcitonin Produced by Medullary Carcinoma of the Thyroid. Proc. Nat. Acad. Sci. U.S.A., 59, (1968): 1216-1222.

Meyer, J. S., and Abdel-Bari, W.: Granules and Thyrocalcitonin-Like Activity in Medullary Carcinoma of the Thyroid Gland. N. Eng. J. Med., 278, (1968): 523-529.

Milhaud, G., and Job, J-C.: Thyrocalcitonin: Effect on Idiopathic Hypercalcemia. Science, 154, (1966): 794-796.

Milhaud, G., and Moukhtar, M. S.: *Hypophysectomie et thyrocalcitonine.* C. R. Acad. Sci., Paris, 260, (1965): 3179-3182.

Milhaud, G., and Moukhtar, M. S.: Thyrocalcitonin: Effects on Calcium Kinetics in Rats. Proc. Soc. Exp. Biol. Med., 123, (1966a): 207-209.

Milhaud, G., and Moukhtar, M. S.: Antagonistic and Synergistic Actions of Thyrocalcitonin and Parathyroid Hormone on the Levels of Calcium and Phosphate in the Rat. Nature, 211, (1966b): 1186-1187.

Mishra, R. K.: Studies on Experimental Magnesium Deficiency in the Albino Rat. 1. Functional and Morphologic Changes Associated with Low Intake of Mg. Rev. Canadienne Biol., 19, (1960): 122-135.

Montgomery, R. D.: Magnesium Metabolism in Infantile Protein Malnutrition. Lancet, II, (1960): 74-76.

Munson, P. L., Hirsch, P. F., Brewer, H. B., Reisfeld, R. A., Cooper, C. W., Wästhed, A. B., Orimo, H., and Potts, J. T., Jr.: "Thyrocalcitonin", in Recent Prog. in Horm. Res., 24, (1968): 589-650.

Neher, R., Riniker, B., Zuber, H., Rittel, W., and Kahnt, F. W.: Thyrocalcitonin. II. Structure of α-thyrocalcitonin. Helv. Chim. Acta, 51, (1968a): 917-924.

Neher, R., Riniker, B., Rittel, W., and Zuber, H.: Human Calcitonin. III. Structure of Calcitonin M and D. Helv. Chim. Acta, 51, (1968b): 1900-1905.

Onderscheka, K., Laber, B., Szekely, H., and Binder, K.: *Chronischer Magnesiummangel bei Kühen.* Wein. Tierartzl. Monatsschr., 54, (1967): 219-236.

Paunier, L., Radde, I. C., Kooh, S. W., Conen, P. E., and Fraser, D.: Primary Hypomagnesemia with Secondary Hypocalcemia in an Infant. Pediatrics, 41, (1968): 385-402.

Potts, J. T., Jr., Niall, H. D., Keutmann, H. T., Brewer, H. B., Jr., and Deftos, L. J.: The Amino Acid Sequence of Porcine Thyrocalcitonin. Proc. Nat. Acad. Sci. U.S.A., 59, (1968): 1321-1328.

Rasmussen, H., and Tenenhouse, A.: Thyrocalcitonin, Osteoporosis, and Osteolysis. Am. J. Med., 43, (1967): 711-726.

Rasquin, P., and Rosenbloom, L.: Endocrine Imbalance and Tissue Hyperplasia in Teleosts Maintained in Darkness. Bull. Am. Mus. Nat. Hist., 104, (1954): 359-426.

Rittel, W., Brugger, M., Kamber, B., Riniker, B., and Sieber, P.: Thyrocalcitonin. III. Synthesis of α-thyrocalcitonin. Helv. Chim. Acta, 51, (1968): 924-928.

Robinson, C. J., Martin, T. J., and MacIntyre, I.: Phosphaturic Effect of Thyrocalcitonin. Lancet, II, (1966): 83-84.

Sanderson, P. H., Marshall, F., and Wilson, R. E.: Calcium and Phosphorus Homeostasis in the Parathyroidectomized Dog; Evaluation by Means of EDTA and Calcium Tolerance Tests. J. Clin. Invest., 39, (1960): 662-670.

Schour, I.: *Noyes' Oral Histology and Embryology.* 7th ed. Lea & Febiger, Philadelphia, Pa., 1953.

Schrader, G. A., Prickett, C. O., and Salmon, W. D.: Symptomatology and Pathology of Potassium and Magnesium Deficiencies in the Rat. J. Nutr., 14, (1937): 85-109.

Sieber, P., Brugger, M., Kamber, B., Riniker, B., and Wittel, W.: Human Calcitonin. IV. Synthesis of Calcitonin M. Helv. Chim. Acta, 51, (1968): 2057-2061.

Sjollema, B.: Nutritional and Metabolic Disorders in Cattle. Nutr. Abstr. and Rev., 1, (1932): 621-632.

Smith, B. S. W., and Nisbet, D. I.: Biochemical and Pathological Studies on Magnesium Deficiency in the Rat. I. Young Animals. J. Comp. Path., 78, (1968): 149-159.

Steel, R. G. D., and Torrie, J. H.: *Principles and Procedures of Statistics.* McGraw-Hill, New York, 1960.

Tufts, E. V., and Greenberg, D. M.: The Biochemistry of Magnesium Deficiency. I. Chemical Changes Resulting from Magnesium Deprivation. J. Biol. Chem., 122, (1938): 693-714.

Wacker, W. E. C., and Parisi, A. F.: Magnesium Metabolism. N. Eng. J. Med., 278, (1968): 658-663, 712-717, 772-776.

Wadkins, C. L.: Experimental Factors that Influence Collagen Calcification *in vitro*. Calc. Tiss. Res., 2, (1968): 214-228.

Walker, D. G.: Elevated Bone Collagenolytic Activity and Hyperplasia of Parafollicular Light Cells of the Thyroid Gland in Parathormone-Treated Grey-Lethal Mice. Zeitschr. für Zellforsch., 72, (1966): 100-124.

Wase, A. W., Solewski, J., Rickes, E., and Seidenberg, J.: Action of Thyrocalcitonin on Bone. Nature, 214, (1967): 388-389.

Watchorn, E., and McCance, R. A.: Subacute Magnesium Deficiency in Rats. Biochem. J., 31, (1937): 1379-1390.

Webster, David, and Frazer, S. C.: Calcitonin and Thyrocalcitonin. Advances in Clin. Chemistry, 10, (1967): 1-44.

White, J. E., and Ahmann, T. M.: Calcitonin Activity in Hereditary Osteopetrosis. J. Clin. Invest., 44, (1965): 1111.

Williams, G. A., Hargis, G. K., Galloway, W. B., and Henderson, W. J.: Evidence for Thyrocalcitonin in Man. Proc. Soc. Exp. Biol. Med., 122, (1966): 1273-1276.

Yamane, G. M., and Singer, L.: The Effect of Subminimal Magnesium on the Syrian Hamster. J. Dental Res., 32, (1953): 708-709.

Young, K., and Booth, E.: Measurement of Serum Calcium and Magnesium by Atomic Absorption Spectrophotometry. Clin. Chem., 13, (1967): 676.

VITA

The author, Daniel Reese Farnell, was born on February 7, 1932, in the port city of Mobile, Alabama. His father, Ralph Farnell, was a tire sales manager. His mother, May Hunter Farnell, born in Cayman Brac, British West Indies, is a retired elementary school teacher.

The author received his elementary and high school education in Mobile, and attended one year at Spring Hill College, a small school operated in Mobile by the Jesuits.

In January of 1951, after attending Auburn University, Auburn, Alabama, for one quarter, the author entered into military service with his National Guard unit, the 31st Infantry "Dixie" Division. After assignments in South Carolina, Texas, and Indiana, he was released from the Army to resume his formal education. He received the degree of D.V.M. in 1957, and immediately joined the staff of Southern Research Institute, Birmingham, Alabama.

In Birmingham, the author was engaged primarily in the screening of drugs and metabolic products of microorganisms for their possible effect in inhibiting the development in animals of transmissible leukemias and other neoplasms.

In 1961, the author returned to Auburn as a Research Fellow, to work toward the M.S. degree and do research in the area of nutrition and cholesterol metabolism. After receiving that degree, he was appointed Associate Professor in the Department of Animal Disease Research at Auburn. His work in that laboratory was in two main areas,

experimental magnesium deficiency and dietary influences on lipid metabolism.

Upon obtaining a Special Fellowship from the National Institute of Arthritis and Metabolic Diseases, the author moved to the Department of Pathology, Michigan State University, for the purpose of doing research and working for the degree of Ph.D. in pathology. The results of this research are the subject of this thesis.

The author is a member of a number of professional and honorary organizations and is a Diplomate of the American College of Laboratory Animal Medicine. He has published about 13 journal articles describing his research.

The author married Carolyn Blanche Williamson of Mobile in 1954. He and his wife have 3 children: Daniel, Jr., age 12 years; Andrea, 11 years; and Cynthia, 3 months.

The author's next move will be to Mississippi State University at Starkville, Mississippi, where he will head the Department of Veterinary Science.