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Effect of Thyroparathyroidectomy on Sutural Growth: A Radioautographic Study

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EFFECT OF THYROPARATHYROIDECTOMY ON SUTURAL GROWTH

A RADIOAUTOGRAPHIC STUDY

By

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Dedicated

To my parents:

Jose L. deSilva Boullosa M.D.

Oralia D. de deSilva

Whose loving devotion, encouragement and sacrifices made possible the completion of my education

To

Alfonso deSilva Boullosa D.D.S.

In appreciation for his inestimable advise and help in the trajectory of my dental education

And

To the memory of my grandmother

Ma. de la Luz Boullosa

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BIOGRAPHY

Jose Luis deSilva Davila was born in Palau, Coahuila, Mexico on July 14, 1950.

He graduated from the "Escuela Preparatoria Varonil de la Universidad Autonoma de Guadalajara", Mexico, where he received the degree of Bachelor of Science in July, 1968.

Inmediately after, he enrolled in the Scool of Dentistry of the Universidad Autonoma de Guadalajara. He completed his dental education in June, 1973. He served one year at the "Centro de Salud No.3" and at the "Hospital Infantil Guadalupano", Guadalajara, Mexico, in fulfillment of the compulsory social service.

After his release from this service, he practiced general dentistry and was a member of the staffs of the "Hospital Infantil Guadalupano" and the "Hospital Maternidad Catalina", in Guadalajara, Mexico, until May, 1975. He obtained his D.D.S. degree in June, 1975.

In July, 1975, he began graduate studies in the Department of Oral Biology and postgraduate studies in the Department of Orthodontics at Loyola University School of Dentistry in Maywood, Ill., U.S.A.

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CHAPTER I

INTRODUCTION

Growth of the cranifacial complex is regulated by a combination of controling mechanisms, that include genetic and environmental factors (Moyers, 1974).

Among those genetically controlled mechanisms, the endocrine influence on growth of the craniofacial complex is of great importance in the atainment of adult adequate proportions. Growth hormone as well as thyroxine are of prime importance in growth, development and maturation.

The influence of the thyroid hormones upon many tissues have been demonstrated (Primack, 1970; Leathem, 1953), and histological findings discussed (Simpsom, 1950; Sokoloff, 1968). However, little reference to what the thyroid hormones participation in growth at the suture has been made.

It is the purpose of this study, to analize the histological findings in the absence of thyroid hormones with the intention of contributing some evidence to the knowledge of craniofacial growth. It will be demonstrated that thyroparathyroidectomy severely affects growth at the sutural area.

CHAPTER II

REVIEW OF THE LITERATURE

A. Origin and Anatomy of the Thyroid Gland.

Tondury and Kistler (1974) reported that in the human, the thyroid gland begins very early in embrionic life. It begins as an outpocketting formed in the floor of the oral cavity. Later it decends caudally but remains attached to the floor of the pharinx by the thyroglosal duct. During this decensus, the original bud becomes bilobed and differentiates into a mass of irregullarly arranged apithelial cord, which later form the isthmus and the lateral lobes of the gland

Hummel et al. (1966) in a review of anatomy, described the thyroid gland in the rat. The rat thyroid, like in the human, consists of two elongated oval lobes, one on either side of the trachea, their porterior poles are joined by a thin isthmus crossing the trachea ventrally. The lobes, buried under the muscles of the neck region, are richly vascularized and made up of groups of hollow spheres often visible macroscopically. The lobes extend anteriorly, as far as the cricoid cartilage of the larinx and posteriorly over the first three or four tracheal rings.

The position of the parathyroids, on the other hand, as well as the number of lobes is variable, although a single lobe lies just under the capsule near the dorsolateral border of each lobe of the thyroid. Two members of a pair are seldom at the same anteroposterior level; sometimes one or both may be posterior to the thyroid; they

may be deeply embedded in the thyroid tissue and there may be more than two.

B. Thyroidal Iodine Uptake.

Besides the kidneys and parotid gland, the thyroids are the only organs clearing significant amounts of iodide from the blood. Wolf (1964) reviewed the mechanisms of thyroidal iodine uptake. Iodide is actively transported across the basal thyroid cell membrane against an electrochemical concentration gradient. Iodide transport requires energy which is supplied by ATP.

Independently of these processes, thyroglobulin molecule precursors are synthesized on the ribosomes of the granular endoplasmic reticulum. They are carried within small vesicles to the golgi region where they seem to be coupled to carbohydrates (Nadler, 1964).

The vesicles then reach the apical surface of the epithelial cell and their membrane fuses with the plasma membrane. Iodination of the thyroglobulin-bound hormone molecules takes place again at the apical surface of the cells, where colloid droplets of varying size are found to be fagozytized.

After breakdown of the thyroglobulin by the hydrolitic enzymes, the hormones thyroxine and triiodothyronine are carried in molecular form to the basal portion of the epithelial cells and reach the capillary network and the termination of the capillary vessels.

C. Thyroid Hormones.

Gross et al. (1950) discovered the presence of some iodinated aminoacids in the thyroid and plasma with chromatographic properties similar to thyroxine. Gross and Pitt-Rivers (1952), identified 3-5-3-triiodothyronine, aminoacid with only three iodide atoms and metabolic

effect. Furthermore, they reported its activity to be five times as great as that of thyroxine in experimental animals.

Braveman (1970) was able to demonstrate peripheral deodination of thyroxine. Athyreotic patients given with supplements of thyroxine showed appreciable levels of triiodothyronine in their plasma. He belived however, that thyroxine may have its own metabolic effect without being converted first to triiodothyronine.

D. Hormone Release and Secretory Rate.

Thyroxine (T^4) and triiodothyronine (T^3) are stored in the colloid of the thyroid glands as peptide-linked aminoacids of thyroglobulin. When hormone is to be released, small droplets of the colloid are engulfed by streamers growing from the apical cell membrane. The droplets move into the cell by a process called endocytosis.

The colloid droplets then move toward the basal cell pole, fusing en route with primary lysosomes. These particles are thought to contain the proteolytic enzymes necessary for the hydrolysis of thyroglobulin. The thyroxine and triiodothyronine thus liberated diffuse into the blood, while mono and diiodothyrosine are inmediately deodinated within the gland by a potent and quite specific deodinase, as shown by Dumas (1973).

E. Pituitary Control of the Thyroid.

One of the functions of the hypothalamus is to maintain the control of a normal concentration of thyroid hormones in the blood stream. In response to neural stimulus, the hypothalamus secretes TRH (thyrotropin-releasing hormone), which reaches the anterior pituitary via a small sistem of portal vessels and produce a rapid release of

TSH (thyroid-stimulant hormone). This hormone which is produced in the basophil cells of the anterior pituitary affect numerous parameters of the thyroid gland within minutes, such as stimulation of colloid endocytosis, glucose oxidation, phosphate incorporation and RNA synthesis. Also, It enhances synthesis of thyroglobulin and the incorporation of iodide into organic compounds (Labhart, 1974).

F. Parathyroids.

The action of the parathyroid hormone is to increase the movilization of calcium from the bones and thus to a rise of the serum calcium concentration. At the same time, PTH promotes the excretion of phosphate through the kidneys and thus to a fall in the serum phosphate concentration. The two actions can not be separated.

PTH concentration in the plasma varies inversely to the serum calcium and magnesium concentration. The ionic calcium concentration rather than the total calcium serum regulates the PTH secretion. The central nervous system and the pituitary gland have no effects on the parathyroids activity.

G. Sites of Action of PTH.

Belager (1968) and Feinblatt (1970), reported that in bones, PTH causes an initial fall followed by a rise in calcium concentration in the plasma. This is caused primarily by the increased movilization of calcium from the bones and an increase in bone resorption with an increase in osteoclastic and osteocytic activity. It promotes the release of calcium from around the osteocytes which are then surrounded by a halo.

PTH causes an increase in alkaline phosphatase in osteocytes.

Pyrophosphates protect bone crystals from dissolution in vitro. Fleisch (1970), and Russel (1968), suggested that the alkaline phosphatase may be a pyrophosphatase, which would stimulate the metabolism of pyrophosphates and the dissolution of bone minerals under the influence of PTH.

The promotion of osteolysis via the osteoclast is one of the chief actions of parathyroid hormone. There is an increase in bone breakdown caused by an increase of the number of osteoclasts and in the amounts of acid phosphatase release from osteoclasts (Reynolds and Dingle, 1970).

Young (1964) showed that the action of PTH on the bone consists predominantly in an increase in the mitotic rate followed by an increase in the number of osteoclasts. An increased incorporation of thymidine into bone cells in vitro indicates a new synthesis of DNA and more frequent cell divisions.

McDonald (1965) reported that PTH not only induce a release of calcium from the bones but also leads simultaneusly to desintegration of bone matrix as indicated by the increased excretion of hydroxyproline.

Fleisch (1966) demonstrated that the dissolution of apatite into calcium and phosphate is promoted by the destruction of a protective layer of pyrophosphate, presumably under the influence of a pyrophosphatase.

The evidence of any action of PTH on bone formation is rather contradictory. Gaillard (1961 and 1967) showed that following the administration of PTH to a variety of species, there is a rapid depression on the metabolic activity of the osteoblasts. Some depression on

glycine incorporation was shown by Flanegan and Nichols (1965). Moreover, Bingham (1969) found a depression of RNA synthesis in vivo in the bones of young rats.

However, there is some evidence of certain anabolic effects. Bingham (1969) also found that after 24 hours, there was an increase rather than depression of RNA in both osteoblasts and preosteoblasts.

Kalu et al. (1970) further demonstrated increased matrix formation, shown by an increased incorporation of ^{3}H-proline, and increased mineralization after 21 days of treatment of thyroparathyroidectomized rats with 50 units of PTH.

H. Calcitonin.

Calcitonin was first extracted by Hirsch (1963). It is secreted by the parafolicular or C cells of the thyroid gland. Its regulation is simultaneous to that of PTH. Calcium and magnesium stimulate the secretion of calcitonin (CT) directly without the interposition of a feedback loop such as the pituitary or the central nervous system. It is a polipeptide hormone which causes the calcium and potassium in the serum to fall.

There is no fall in CT concentration in the plasma of thyroparathyroidectomized animals since this hormone is also synthesized in other organs, as shown by gudmunson (1969), and Kaplan (1970).

Labhart (1974) in a review of the calcitonin activity synthesized that its most important action is the inhibition of bone resorption, and that stimulation or inhibition of bone formation had not been definitely found.

I. Mode of Action of Thyroid Hormones.

The most conspicuos action of the thyroid hormones, as shown by Barker (1952) is their ability to increase oxigen consumption and heat production, usually measured as an increase in basal metabolic rate. He demonstrated this effect to be true in liver, kidney and muscle. However, other tissues like gonads, genital organs, brain and lymphatic tissue did not respond to this action.

It is recognized that thyroxine stimulates protein synthesis in mitochondria, as shown by the incorporation of radioactive aminoacids into mitochondrial protein (Tapley et al., 1967).

Griswold and Cohen (1972) demonstrated that thyroxine was necessary in the induction for urea synthesis in the liver of the tadpole. Furthermore, that its site of action was the cell nucleus, where it stimulates the synthesis of DNA-dependent RNA-polymerase, an enzyme necessary for the production of mRNA.

Sokoloff (1968) reported the stimulatory effect of thyroxine on microsomal protein synthesis. This was the result of microsomal function, dependent on mitochondria or an ATP generating system and independent transcription of mRNA synthesis; it preceds any change in mRNA concentration. It was concluded that it must have been a hormone action at the translational (ribosomal) level.

Another effect was described by Sokoloff (1964), and Tata (1966, 1967 and 1968). This was a late effect on protein synthesis on the liver, independent of the presence of mitochondria, but required the synthesis of mRNA. This was thought to be due to a hormone effect on the transcription of RNA in the cell nucleus.

J. Sutures and Bone Structure.

There are two types of bone proliferation, endochondral bone formation, in which cartilaginous cells develop from undifferentiated mesenchymal cells. This chondrocytes grow interstitially and later in their development they differentiate and undergo degenerative changes, they are resorbed and replaced by bone.

The second type of bone formation is the membranous, in which the osteoblasts originate directly from the connective tissue. This type of bone growth can only increase in size by apposition of new layers of bone on the existing bone surface.

Enlow (1974) described the basic structure of the sutures and compared the osteogenic process occurring at the periosteum with that occurring at the suture, since the latter is an inward reflexion of the periosteal membrane. Growth at this site occurs by appositional growth, adding new layers of bone on the sutural edge.

He recognized three basic layers on each side of the suture, the bone edge itself, the cambial layer and the fibrous capsule. However, he added, that some sutures might have another layer of loosely arranged fibers located between the two dense fibered capsular layers.

Formation of bone is regulated by the presence of osteoblasts and osteocytes, which originate directly from undifferentiated mesenchymal cells. The cytoplasm of the osteoblasts is rich in ribonucleic acid, which regulates glycoprotein synthesis and certain enzymes such as alkaline and acid phosphatases.

The osteoblast secrete the organic bone matrix, consisting of collagen in 90-95 % and chondroitin sulfate. Collagen is a protein containing aminoacids such as glycine (30%), proline (12%), and hydroxy-

proline (10%). The osteoblasts are responsible for the synthesis of collagen. It occurs by taking up free aminoacids existing in the media and depositing them in the ribosomes where protocollagen is formed. Protocollagen in turn is secreted in the intercellular fluid where it is transformed first into "neutral salt-soluble", and then into "insoluble" collagen fibrills which are then connected to form a network Prockop, 1967).

In addition to collagen, bone matrix is formed in lesser proportion by the ground substance (1-2%), made up of muchopolysacharides such as chondroitin sulfate which bind to collagen hydroxyl group to form glycoproteins.

Calcium and phosphate in the form of amorphus calcium phosphate and hydroxyapatite are subsequently deposited in the matrix of collagen and chondroitin sulfate by a process not yet well understood (Labhart, 1974).

K. Effects of Thyroidectomy on Growth.

Thyroid insufficiency causes a marked retardation on growth and delayed maturation of the skeletal system as is shown by Salmon (1938), Scow (1945), and Leathem (1953).

Simpsom et al. (1950) reported that thyroidectomized animals failed to advance in skeletal age and compared the skeletons of thyroidectomized animals with those hypophysectomized, in their size and unexpanded epiphyseal centers. However, certain differences were noted. In the thyroidectomized, some activity persisted in the cartilage plates and some erosion was still occurring, whereas the hypophysectomized animals, cartilage plate showed almost no activity and it was reduced in width at once. No erosion occurs as cartilage is sealed from marrow by

bone.

The bones of the thyroidectomized rats also differed from those of hypophysectomized in that primary trabeculae remained continuos with the sealing lamina of bone.

Boere and Garenstrom (1943) reported that skeletal growth is stimulated by growth hormone as well as by thyroxine. Bone length and bone width increases in hypophysectomized rats by administration of growth hormone. Thyroxine merely enlarges bone lenght.

There appears to be a potentiation as a result of the interaction of these two hormones, as has been shown by Groot (1963), Simpson et al. (1950) and Pannain (1966).

Bone growth is apparently induced by each hormone in a different way. Thyroxine accelerates bone formation, which is made possible by a constant renewal of cartilage, induced by growth hormone (Simpson et al., 1950).

L Kinetic Studies of Bone.

Radioactive isotopes have been widely used in the study of bone kinetics. Kinetics depicts the measurement of movement of isotopes which are exchanged and incorporated into bone.

Young (1962) utilized tritiated glycine in an autoradiographic study of bone formation in the calvaria of young rats. He reported that activity of the glycine-H^3 at the parietal suture was mainly extranuclear and apparently cytoplasmatic of osteoblasts and the bone surface. Osteocytes and fibroblasts in lesser degree, and no uptake by the osteoclasts.

The introduction of thymidine labeled by tritium (Verley and

Hunebelle, 1957; Firket and Verley, 1958; and Taylor et al., 1957), introduced the opportunity to investigate the proliferation and cell division in a more accurate way.

The information for the reproduction of every cell is stored in the form of genes within chromosomes in the nucleus of every cell. Chemically genes are long strands of desoxyribonucleic acid (DNA), which contain the information for protein synthesis, in the form of a variable sequence of four bases, adenine, guanine, cytosine and thymine. RNA on the other hand, has the same three first bases, but uracil substitutes thymine. Thus, the presence of thymine surely identifies the existence of DNA.

When labeled thymidine is injected into an animal, the cells that are in the process of division at that time take up the labeled thymidine and incorporate it into their nuclear DNA. The percentage of cells bearing the label tells what proportion of cells are synthesizing DNA at a given time (thymidine index). This is the rate of cell division, or how fast the cell population is proliferating (Taylor et al., 1957).

Kember (1960) in an autoradiographic study with tritiated thymidine described the pattern of labeling of endochondral cells in the epyphyseal cartilage plate in the tibia of young rats. He reported that the mesenchymal cells found inmediately below the cartilage plate and throughout the rest of the metaphysis was where the highest proportion of tritiated thymidine labeling was found.

Young (1962) calculated the cell generation time for the metaphysis, endosteum and periosteum in the growing rat tibia. He found them to be 35, 57 and 114 hrs. respectively. At one hour after injec-

tion he observed only mesenchymal cells revealed an uptake of tritiated thymidine.

M. Pathway of Incorporation and Degradation of Tritiated Thymidine.

Tritiated thymidine once injected into the rat is carried by the blood vessels into the capillaries of the suture, where it diffuses into the intercellular spaces of the connective tissue. The cellular elements of the connective tissue pick up the labeled thymidine in their process of synthesizing elements for their reproduction. Tritiated thymidine thus enters the preosteoblast by this mechanism which occurs at a rather rapid rate.

It is incorporated into DNA by a sequence of phosphorylation steps through thymidine monophosphate, thymidine diphosphate and thymidine triphosphate; followed by the assembly of thymidine triphosphate, together with other nucleoside triphosphates, into DNA. Some enzymes are involved in this process, thymidine kinase, thymidine monophosphate kinase and thymidine diphosphate kinase, as shown by Cleaver (1967).

The path of degradation consists of the cleavage of the glycosidic bond in the tritiated thymidine to form thymine and desoxyribose-1-P by a phosphorylase. Thymine is converted to dihydrothymine, B-ureidoisobutiric acid (BUIB), B-aminoisobutiric acid (BAIB), to carbon dioxide, amonia and water (Fink et al., 1956b; Potter, 1959; Armstrong et al., 1963). The final degradation steps to BAIB, CO_2, etc., are belived to occur in the liver, splen and kidneys, whereas the earlier steps, particularly the one involving the phosphorylase occur in many tissues (Friedkin and Roberts, 1954; Rubini et al., 1960; Marsh and Perry, 1964b).

N. Radioautographic technique.

Kopriwa and Leblond (1962) described some improvements in the coating technique for autoradiography and staining. Tritium, a radioactive isotope of hydrogen, with three times the mass of ordinary hydrogen, emits beta particles with short range (1 micron). The short range allows for autoradiography of tissues and the resolution is excellent. However, it requires that the labeled structures lie in the upper 1 or 2 microns of the tissue next to the emulsion in order to produce an image.

When the crystals of silver bromide embedded in gelatin of the emulsion are struck by these particles, they are ionized with the result that a latent image forms. The chemical action of a developer can then reduce the bromide to silver atoms which are visible under the light microscope once the tissue slides are developed and fixed. Each little aggregate of reduced silver atom appears as a black dot directly above the labeled structure.

Every cell during part of its differentiation goes through two phases, mitosis and interphase. Howard and Pelc (1953) suggested that every cell goes through a progression cycle that could be represented as sectors of a clock's face. Mitosis will represent but a small part of that cycle. At this period, no DNA, little proteins and sometimes no RNA are produced. After mitosis, the cell goes into a period called G_1, then S, G_2 and mitosis again. During S phase, DNA, RNA and proteins are built up. In the course of phases G_1 and G_2, RNA and proteins are being synthesizes but no DNA synthesis occurs (Harbers et al., 1968).

CHAPTER III

METHODS AND MATERIALS

For the purpose of this study, twenty seven one month old male albino rats were used. The animals were purchased from The Hormone Assay Laboratories Inc., Chicago, Ill.

Fourteen of them had the thyroid and parathyroid glands removed when they were thirty days old. The remaining thirteen unoperated animals were used as controls.

At their arrival at the animal laboratory facility, the animals were weighed, marked and placed in separate cages. The control animals were given a standard diet of Purina Rat Chow[1] which was supplied with water "ad libitum". The experimental animals were provided with the same diet and 1 % of calcium chloride diluted in water "ad libitum". This was done as a precaution to prevent tetany as a consequence of the parathyroidectomy. Simpson et al. (1950) reported that rats are very resistant to tetany if they are on a high calcium diet.

The animals were kept for seven days. Then, both the controls and the experimental animals were weighed again as to record and assess any difference in their weight. They were injected intraperitoneally with tritiated thymidine[2] at a doses of 0.7 uc (microcuries) per gram of animal weight (specific activity 1.9 c/mM at a concentration of 1 mc/ml.). One hour later they were sacrificed by ether inhalation and

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1- Ralston Purina Co., St. Louis, Mo.

2- New England Nuclear, Boston, Mass.

the parietal bones were removed from their calvarias. Then, they were trimmed as to obtain 1 cm^2 of bone section with the interparietal suture located at the middle and fixed in 10 % formaline.

The samples of tissue were decalcified in formic acid and citric acid 50:50 and prepared to be embedded in pareffin. Each group, experimental and control were places in two separate blocks of paraffin. Ten sections 6 u. thick were cut from each specimen by microtome[3] and extended on a glass slide.

The slides were submerged in liquid emulsion NTB-2[(4)], drained, and placed in light tight boxes for ten days in a freezer at $0^{o}C$. Then, they were developed, fixed and stained with nuclear red and indigo carmin.

The prepared slides were placed under the light microscope and the sutural areas of both, the experimental and control animals were evaluated.

Utilizing an eyepiece with a reticular representing 100 u^2 [(5)], the cellular densities for the sutural randomly selected area of each animal were averaged and the results reported. The mean cellular density of the experimental and that of the controls were recorded.

The labeling index of both groups was calculated by counting the number of labeled nuclei per 100 u^2. Since the mean cellular density was previously recorded, the number of reticulars necessary to make up 10,000 cells on either group was calculated.

3- "820" Microtome, American Optical Co., Buffalo, N.Y.

4- Eastman Kodak Co., Rochester, N.Y.

5- American Optical Co., Buffalo, N.Y.

CHAPTER IV

RESULTS

Both groups of animals stayed apparently healthy and no complications developed during the experimental period of one week.

The water intake and food consumption of the thyroparathyroidectomized animals were remarkably reduced. They were lethargic and much less active than the control animals.

At the end of the week, the experimental animals had failed to grow in size and body volume as much as the controls. The difference in weight gains of the two groups is shown in table I. The mean weight gain for the thyroparathyroidectomized animals was found to be 18.08 grs., whereas the controls were found to be 48.41 grs. The statistical analysis showed this difference to be significant at the .01 level of confidence.

Utilizing the low power of the microscope (40X), no significant difference was noted in any group, as the bone thickness and the width of the suture appeared to be the same.

With the aid of a higher power (100X), some differences were observed. The endocranial plate of bone on the experimental group showed a more irregular contour, with the marrow spaces of the bone often communicating with the endocranial lining of osteoblasts, in comparison with the normal controls which showed a well defined and evenly calcified endocranial plate (Fig. 1 and 2).

The lacunae on the areas adjacent to the sutural edge appeared again to be larger in the experimental animals than in the controls.

The cellular density of the sutures was calculated for both groups and it is shown in table II. The experimental animals showed a marked reduction in cellular density per reticular (100 u^2). This difference was found to be statistically significant at the .01 level of confidence by means of the student "T" test.

Also the cellular elements of the proliferative zone of the thyroparathyroidectomized animals appeared to be larger in size and more collagen existed between them, when compared to normal animals (Fig. 3 and 4).

Most of the cells found to be bearing the thymidine label were fibroblast-like cells and probably preosteoblasts. However, no osteoblasts were observed to be labeled.

The number of tritiated thymidine labeled cells counted per reticular is shown in table III. The ratio of labeled cells for the experimental group was 146/10,000 counted cells. The ratio for the control was 213/10,000 labeled-non labeled cells, which was proven to be statistically different again at the .01 level of confidence by means of the X^2 method.

TABLE I

WEIGHT OF THYROPARATHYROIDECTOMIZED AND CONTROL ANIMALS

(Expressed in grams)

Experimental

Before	After	Net Wt. Gain	
73	74	1	
73	85.5	12.5	
73.5	88	14.5	
74.5	89	14.5	
76	89.5	13.5	Mean: 18.08
76	90	14	Variance: 79.5
78	92	14	St. Dev.: 8.91
78	93	15	
78	93.5	15.5	
78.8	100	21.2	
79.5	102.5	23	
79.5	106.5	27	
82	113.5	31.5	
82	118	36	

Control

Before	After	Net Wt. Gain	
68.3	116	47.7	
70.5	116	45.5	
71	117	46	
72	117.5	45.5	
72	118	46	Mean: 48.41
72.8	119	46.2	Variance: 12.27
74	120	46	St. Dev.: 3.5
74.5	120.5	46	
74.4	125	50.5	
75.5	125.5	50	
76	128.5	52.5	
78	135	57	
86.5	137.5	50.5	

$P < .01$

TABLE 2

CELLULAR DENSITY PER 100 u^2

Experimental	Control
93	120
95	118
94	114
105	114
103	135
96	106
124	113
123	108
109	126
113	124
107	108
	138
	125
Mean 105.63	119.15
S.D. ± 11	10.1
Variance 121.4	102.14

$P < .01$

TABLE 3

NUMBER OF LABELED CELLS PER RETICULAR

($100\ u^2$)

Experimental

3	1	0	2	2	2
4	1	2	2	1	3
1	1	1	1	2	2
2	1	0	0	1	2
1	0	0	2	0	3
0	1	3	0	3	2
1	3	0	0	0	1
0	0	0	0	4	2
2	0	1	1	2	4
1	3	2	3	1	2
2	3	2	0	2	2
2	2	2	0	0	1
0	1	0	2	5	1
2	2	1	2	0	3
2	2	1	8	2	
3	2	1	1	2	

No. of Reticulars:
94
No. of Labeled Cells:
146/10,000

Control

1	2	1	5	1	7
2	1	1	2	0	4
6	2	5	8	3	15
3	5	0	2	3	2
2	1	2	2	1	17
2	0	0	1	1	2
0	1	1	0	2	7
1	1	2	5	4	5
1	0	2	0	5	6
0	2	1	4	2	2
2	3	2	3	2	1
2	0	1	3	5	3
0	1	1	4	2	0
2	2	1	1	3	3

No. of Reticulars
84
No. of Labeled Cells
213/10,000

$P < .01$

CHAPTER V

DISCUSSION

This study shows that the absence of thyroid hormones produce a decrease in sutural growth in thyroparathyroidectomized animals, as measured by the cellular density and labeling index of its connective tissue.

Thyroparathyroidectomy causes a reduced cellular density in the sutural area of the parietal bones. This effect is the result of the decreased availability of the thyroid hormones on the osteoprogenitor cells of the connective tissue of the suture. The reduced cellular proliferation is brought about as a result of the failure of the cellular elements to reproduce.

The decreased labeling index in the experimental animals explain the reduced cellular density at the sutural area. The reduced incorporation of thymidine into the cell impairs the process of synthesis of DNA, which in turn reduce mitotic activity and causes a decrease in cellular density.

The energy requirements for growth are derived from carbohydrate metabolism which is moderated by thyroid hormones.

In the process of synthesis of nucleotides, a negative copy with the complimentary base sequence must be first obtained from the existing DNA of the particular gene to be replicated. From here, several steps have to occur before the cycle is completed, transcription of DNA into mRNA, rRNA or tRNA; translation of RNA into proteins; and uptake of the particular aminoacids from the extracellular fluid.

All these biosynthetic processes require energy that should be supplied by ATP. ATP is necessary to produce the phosphate diester linkages between the individual nucleotides to form nucleotide-triphosphate which are assemblied into DNA.

One of the sites of action of thyroxine is the mitochondria (Sokoloff, 1968). an organelle responsible for the oxidation of many compounds. This oxidation yields the high energy phosphates which then are used in all processes of oxidative phosphorilation. Part of this energy is obtained through the oxidation of glucose in the Embden-Meyerhof Cycle and in the Citric Acid Cycle (Krebbs Cycle), which is the final pathway of oxidation of carbohydrates, fats and proteins.

Also, thyroxine has a direct effect on the adenyl cyclase system (Levey, 1969), which activates cyclic AMP involved in the process of glycogenolysis. This activity serves to provide glucose to proliferating cells during growth, development and homeostasis.

Thus, metabolism of the connective tissue may be impaired by several mechanisms. Availability of glucose is reduced since its absorption from the intestines is diminished in the absence of thyroid hormones. Glucose oxidation at mitochondrial level may be equally impaired even in the reduced presence of this carbohydrate, with the resultant of less production of ATP.

If the presence of specific receptors in the osteoprogenitor cells could be demonstrated, one could assume that there is a direct depressant effect in the absence of thyroid hormones on the connective tissue. The second explanation for the reduced cellular activity on the suture could be the general depression of the basal metabolic rate. The third possibility is that these two mechanisms, the direct

depression on the connective tissue and the reduction of the basal metabolic are acting in conjunction to produce this effect.

It can be explained, at least in part, how in the absence of thyroid hormones, the mechanisms of energy production by glucose metabolism may be severely impaired. The cellular elements not having sufficient energy are uncapable of synthesizing DNA necessary for their replication, thus, they fail to reproduce.

Bone formation also is affected in the way that the operated animals showed a more irregular bone contour and apposition seemed to be reduced in some areas. We can assume that the mechanisms of protein synthesis, in this case, synthesis of collagen, glycoproteins and chondroitin sulfate, involved in the formation of bone matrix and ground substance, may be profoundly altered in this condition, since it has been shown by Sokoloff (1964), and Tata (1966, 1967 and 1968), that protein synthesis itself is controled by thyroid hormones. One effect is at a translational stage, independent of mRNA synthesis. Another on the transcription of RNA, dependent of the synthesis of mRNA. Also some stimulatory effects on protein synthesis in mitochondria have been discussed by Primack (1970), and Volfin (1969).

The reduced food consumption of thyroparathyroidectomized animals could contribute even further to a low calorigenic content of glucose, proteins and fatty acids in their blood. This condition would reduce the energy supply to growing cells, thus causing a decreased metabolic status of the organism. In this way one can explain the lethargic appearence of these operated animals.

However, thyroparathyroidectomized animals showed slight though positive growth in spite of the lack of thyroid hormones. This fact is

supported by the observation of labeled cells on the sutural area of the parietal bones of these animals.

Since the hypophysis is intact in thyroparathyroidectomized animals, the action of STH is still expected to occur. Thus, STH would account for the observed growth of these animals (Simpsom et al., 1950). Growth hormone stimulates protein synthesis and through nitrogen retention promotes a positive nitrogen balance. Also, it facilitates the transport of aminoacids into the cells, where it promotes gluconeogenesis.

Thyroparathyroidectomized animals are undernourished and their metabolic status is rather that of starvation. In this condition, energy requirements are obtained from the breakdown of tissue proteins to aminoacids. These aminoacids are then converted to glucose through gluconeogenesis, promoted by growth hormone.

The production of cortisol by the adrenal medula also promotes protein metabolism in the cell and utilization of lipids. Insulin acts directly on intracellular transport of aminoacids and also facilitates glucose utilization, although this effect is antagonized by growth hormone. Moreover, epinephrine causes an increase in glycogenolysis in the liver, thus increasing the circulating blood glucose.

Even in the absence of thyroid hormones, thyroparathyroidectomized animals utilize the few calorigenic supply still available from their reserves of glycogen, fat and tissue protein through the action of these hormones and fulfill partially their requirements. Thus, glycogenolysis, gluconeogenesis and lipolysis keep the basic metabolic demands of the organism, until all the fuel reserves are exhausted.

According to the findings that the lack of thyroid hormones affect the growth of the suture by reducing the cellular proliferation, one can consider this area as an active site of growth under a well defined endocrine influence. Nevertheless, by no means this endocrine influence should be considered solely responsible for the growth of the desmocranium, since there is an active interaction between the genetically controlled mechanisms of growth and those produced by environmental factors.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Effects of thyroparathyroidectomy were evaluated in the sutural area of the parietal bones in thirty day old albino rats.

Fourteen thyroparathyroidectomized and thirteen normal rats were kept for seven days and their weight gain was recorded. Then, both groups were injected intraperitoneally with tritiated thymidine and sacrificed one hour later.

A section of their calvarias comprising the interparietal suture was removed, histologically prepared and its morphology evaluated. Also, cellular density and labeling index of the thyroparathyroprivated animals were compared to those of the normal controls.

There was a failure of the operated animals to gain weight during the experimental period of one week. Cellular density and labeling index were significantly reduced in the experimental group and some alteration in bone bormation was observed.

The findings observed were explained by the profound disruption that the lack of thyroid hormones bring about to the rat organism. Glucose absorption and utilization may be severely impaired as well as protein synthesis, resulting in a decreased availability of ATP necessary for DNA synthesis.

CHAPTER VII

BIBLIOGRAPHY

Armstrong, M.D., Yates, K., Kakimoto, Y., Taniguchi, K., and Kappe, T.: Excretion of B-aminoisobutiric acid by man. J. Biol. Chem. 238: 1447, 1963.

Barker, S.B., Klitgaard, H.M.: Metabolism of tissues excised from thyroxine injected rats. Amer. J. Phys. 170:81, 1952.

Belanger, L.F., and Rasmusen, H.: Inhibition of osteocytic osteolysis by calcitonin and some anti-growth factors. In: Parathyroid Hormone and Thyrocalcitonin (calcitonin). p.156. Ams. Excerpt. Med. 1968.

Bell, G.H., Davidson, J.N., Emslie-Smith, D.: Textbook of Physiology and Biochemistry. 8th. Ed. Baltimore. Williams and Wilkins, 1972.

Bingham, P.J., Brazell, I.A., and Owen, M.: The effect of parathyroid extract on cellular activity and plasma calcium levels in vivo. J. Endocrinology. 45: 387-400, 1969.

Boere, J.M., and Gaarenstroom, J.H.: Proc. Kon. Ned. Akad. Wet. 52 (1943) 2

Braveman, L.E., Ingbar, S.H., Sterling, K.: Conversion of thyroxine (T^4) to triiodothyronine (T^3) in athyreotic human subjects. J. Clin. Inves. 40: 855, 1970.

Cleaver, J.E.:Thymidine Metabolism and Cell Kinetics. North Holland Pub. Co., (Ams.), John Wiley and Sons Inc. New York. 1967 pp. 43-44, 54.

Dumas, P., Maziere, B., Auttissier, N., Michell, R.: "Specificite de l'iodotyrosine deodinase des microsomes tyroides et hepatiques". Biochem. Biophys. Acta. (Ams) 293:36, 1973.

Enlow, D.: Handbook of Facial Growth. W.B. Saunders Co. Philadelphia, 1975.

Feinblatt, J., Belager, L.F., Ramussen, H.: Effect of phosphate infusion on bone metabolism and parathyroid hormone action. Amer. J. Physiol. 218: 1624, 1970.

Fink, R.H., McGaughey, C., Cline, R.E. and Fink, K.: Metabolism of intermediate pyrimidine reduction products in vitro. J. Biol. Chem. 218: 1, 1956b.

Firket, H., and Verley, W.G.: Autoradiographic visualization of synthesis of desoxiribonucleic acid in tissue culture with tritium labeled thymidine. Nature. 181: 274, 1958.

Flanegan, B., Nichols, G.: Metabolic studies of human bone in vitro, II. Changes in hyperparathyroidism. J. Clin Invest. 44: 1795-1804, 1965.

Fleisch, H., Russel, R.G.G.: Phyrophosphate, poliphosphate and calcium homeostasis. In: Encyclopedia of Pharmacology. H. Rasmussen Ed. New York Pergamen Press. 1970.

_____, Maerki, J., Russel, R.G.G.: Effect of phyrophosphate on dissolution of hydroxiapatite and its possible importance in calcium homeostasis. Proc. Soc. Exp. Biol. New York. 122: 317, 1966

Friedkin, M., Roberts, D.: The enzymatic synthesis of nucleosides. J. Biol. Chem. 207: 245, 1954.

Gaillard, P.G.: Parathyroid and bone tissue culture. In: The Parathyroids. Greep, R.O. and Talmage, R.V., Eds., pp. 20-45. Thomas. Springfield, Ill., 1961.

_____: Bone culture studies with thyrocalcitonin. Proc. K. Ned. Akad. Wet. Ser. C70: 309-20, 1967.

Griswold, M.D., Cohen, P.P.: Alteration of DNA dependent ribonucleic acid polymerase activities in amphibian liver nuclei during thyroxine induced methamorphosis. J. Biol. Chem. 247: 353, 1972.

Groot, C.A.: Tail growth in thyroxine treated hypohysectomized rats as a sensitive criterion for growth hormone activity. Acta Endo. 42: 423, 1963.

Gross, J., Leblond, C.P., Franklin, A.E.: Presence of iodinated amino-acids in unhydrolized thyroid and plasma. Science III: 605, 1950.

_____, Pitt-Rivers, R.: Identification of 3-5-3 triiodothyronine in human plasma. Lancet 1: 439, 1952.

Gudmunson, T.V., Woodhouse, N.J.Y., Osato, T.D., Galante, L., Mathews, E.W., Macintyre, I.: Plasma calcitonin in man. Lancet 1: 443, 1969.

Harbers, E., Domagk, G., Muller, W.: Introduction to Nucleic Acids. Reinhold Book Co., New York and London, 1968.

Harper, W.R.: Review of Physiological Chemistry. 15th. Ed. Lange Med. Pub., Los Altos, Ca.

Harrington, C.R., Barger, G.: Chemistry of thyroxine. I. Insolation of thyroxine from the thyroid gland. Biochem. J. 20: 293, 1926.

_____: Chemistry of thyroxine. III. Constitution and synthesis of thyroxine. Biochem. J. 21: 169, 1927.

Hirsch, P.O., Gauthier, F., Munson, P.L.: Thyroid hypocalcemic principle and recurrent laringeal nerve injury as factors affecting the response to parathyroidectomy in rats. Endoc. 73: 244, 1963.

Howard, A., Pelc, S.R.: Isotopes in Biochemistry. G.E.W. Wolsten Holme Ed. Churchil, London. p. 138.

Hummel, K.P., Richarson, F.L., Fekete, E.: Anatomy, In: Biology of the Laboratory Mouse. The Jackson Lab., Earl L. Green Ed. 1966. pp. 258-59.

Kember, N.F.: Cell division in endochondral ossification. J. Joint Bone Surg. 42-B: 824-39, 1960.

Kalu D.M., Doyle, F.M., Pennock, J., Foster, G.W.: Parathyroid hormone and experimental osteosclerosis. Lancet 1: 1363-6. 1970.

Kaplan, E.L., Arnaud, C.D., Hill, B.J., Peskin, G.W.: Adrenal medullary calcitonin-like factor: a key to multiple endocrine neoplasia type 2 (?). Surgery 68: 146, 1970.

Kopriwa, B., Leblond, C.: Improvements in the coating technique of radioautography. J. Histochem. and Cytochem. 10: 269-284, 1962.

Labhart, A.: Clinical Endocrinology, Theory and Practice. Springer-Verlag. New York, 1974.

Leathem, J.H.: Relationships between the thyroid and protein metabolism. In: Protein Metabolism, Hormones and Growth. W.H. Cole Ed., New Brunswick, N.J. Rutgers Univ. Press. 1953. pp.17

Marsh, J.C., Perry, S.: Biophys. 104: 106, 1964b.

McDonald, J.E., Mamola, N., Hanneman, P.H.: Urinary hydroxiproline excretion as an index for bone resorption. Clin. Res. 13: 329, 1965.

Moyers, R.E.: Handbook of Orthodontics. 3rd. Ed. Year Book Med. Pub. Inc., Chicago, 1974.

Nadler, N.J., Young, B.A., Leblond, C.P., Mitmaker, B.: Elaboration of the thyroglobulin in the thyroid follicle. Endoc. 74: 333-354, 1964.

Pannain, R.: "Efeitos da administracao insulada ou conjunta de hormonio de crescimento e de tiroxina, no crescimento do cranio visceral de ratos hipofisectomizados". Rev. F.O.A. 2: 51, 1966.

Potter, V.R. In: Kinetics of Cellular Proliferation. F. Stohlman Ed. Grune and Stratton. New York, 1959.

Primack, M.P., Buchanan, J.L., Tapley, D.F.: Early stimulation of mitochondrial protein synthesis in livers from triiodothyronine injected mice. Endoc. 87: 1355, 1970.

Prockop, D.J., Kivirikko, K.J.: Relation of hydroxiproline excretion in urine to collagen metabolism. Ann. Inter. Med. 66: 1243, 1967.

Reynolds, J.J.: A sensitive method in vitro for studying the induction and inhibition of bone resorption. Calc. Tissue Res. 4: 339-349, 1970.

Rubini, J.R., Cronkite, E.P., Bond, V.P., Fleidner, G.: The metabolism and fate of Tdr. in man. J. Clin. Invest. 39: 909, 1960.

Russell, R.G.G., Casey, P., Bisaz, S., Fleisch, H.: Further studies on the role of phyrophosphates. In: "Les Tissus Calcifies". p.65, Paris. Soc. d'Ed. d'Einseignement Sup., Bordeaux, 1968-a.

Salmon, T.M.: The effect of the growth rate of thyroparathyroidectomized newborn rats and the subsequent administration of thyroid, parathyroid and hypophysis. Endoc. 23: 446-457, 1938.

Scow, R.O., Simpsom, M.: Thyroidectomy in the newborn rat. Ana. Record 91: 209-225, 1945.

Simpsom, M.E., Asling, C.N., Evans, H.M.: Some endocrine influence on eskeletal growth and differentiation. Yale J. Biol. Med. 23: 1, 1950.

Sokoloff, L., Francis, C.M., Campbell, P.L.: Thyroxine incorporation of aminoacid incorporation into proteins independent of any action on messenger RNA synthesis. Proc. Nat. Acad. of Science. 52: 728, 1964.

_____, Roberts, P.A., Janusla, M.M., Kline, J.E.: Mechanism of stimulation of protein synthesis by thyroid hormones in vivo. Proc. Nat. Acad. of Sc. (Wash.), 60: 652, 1968.

Tapley, D.F., Kimberg, D.V., Buchanan, J.L.: The mitochondrion. New England J. of Med. 276: 1182, 1967.

Tata, J.R., Windell, C.C.: Ribonucleic acid synthesis during the early action of thyroid hormones. Biochem. J. 98: 604, 1966.

_____: Thyroid hormones and the formation and distribution of ribosomes. Acta Endoc. (Kbh,), Suppl. 124 to vol. 56, 1967 p. 141

_____: Ribosomes and the Thyroid Hormones. In: Wirkungs Mechanismen Der Hormone. Berlin-Heidelberg-New York.: Springer 1967.

_____: Hormonal regulation of growth and protein synthesis. Nature. (London), 219: 331, 1968.

Taylor, J., Wood, P., Hughes, W.: The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Nat. Ac. of Sc. 43: 122-128, 1957.

Tondury, G., Kistler, G.: Embriology, Gross Anatomy and Histology of the Thyroid Gland. In: Clinical Endocrinology. A. Labhart. Spring-Verlag. New York 1974. p. 136.

Verley, W.G., Hunebelle, G.P.: Bull. Soc. Chim. Belges 66: 640, 1957.

Volfin, P., Kaplay, S.S., Sanadi, D.R.: Early Effects of thyroxine in vivo on rapidly labeled mitochondrial protein fractions and respiratory control. J. Biol. Chem. 244: 5631, 1969.

Wolf, J.: Transport of iodide and other anions in the thyroid gland. Phys. Rev. 44: 45, 1964.

Young, R.W.: Regional differences in cell generation tissue in growing rat tibiae. Exp. Cell. Res. 26: 562-567, 1962.

_____: Autoradiographic studies on post-natal growth of the skull in young rats injected with tritiated glycine. Anat. Record 143: 1, 1962.

_____: Specialization of Bone Cells. In: Bone Biodinamics. (H.M. Frost. Ed.) Boston. Little Brown, 1964.

FIGURE I Photomicrograph (X100) showing the structure of the parietal bones in an experimental animal.

FIGURE II Photomicrograph (X100) showing the structure of the parietal bones in a control animal

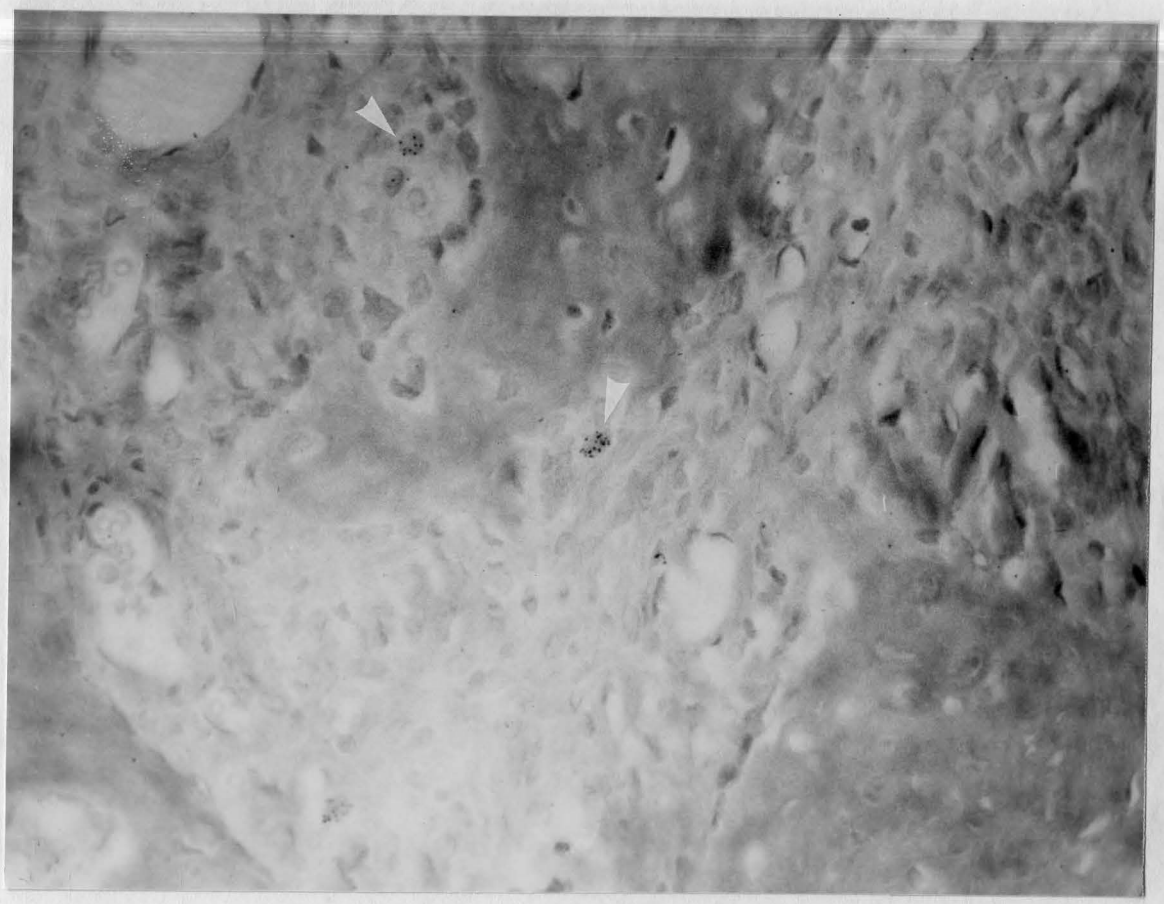

FIGURE III Photomicrograph (X400) of an autoradiogram showing labeled cells in a transverse section of the interparietal suture in an experimental animal.

FIGURE IV Photomicrograph (X400) of an autoradiogram showing labeled cells in a transverse section of the interparietal suture in a control animal.

APPROVAL SHEET

The thesis submitted by Jose L. deSilva D.D.S. has been read and approved by the following committee:

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

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

May 23, 1977
Date

Director's Signature