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The Effects of Tissue Culture Medium (T199) on Perichondrium of the Mandibular Condyle in Rabbits

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THE EFFECTS OF TISSUE CULTURE MEDIUM (T199) ON THE PERICHONDRIUM OF THE MANDIBULAR CONDYLE IN RABBITS

BY

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DEDICATION

To my wife Carmen and sons Mauricio and Alejandro, for their love, devotion and patience.

To my mother Victoria Oteo and Dr. Rafael Ortega, Virginia Ortega and R.P. Oscar Oteo for offering the greatest support and encouragement throughout my life.

To my cousins and friends Rafael, Oscar, Alejandro, Roberto, Virginia and Louis Octavio.

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VITA

The author, Agustin Lopez Oteo, was born on June 21, 1945, in Mexico, D.F. Mexico to Raul Lopez Anitua and Victoria Oteo Thompson.

In 1966, he graduated from Academia Militar Mexico. He began his formal dental studies at the University of Mexico in 1967, and graduated in 1971 with a Doctor of Dental Surgery degree. He worked for 6 years teaching Dental Operatory in Universidad Tecnologica de Mexico and private practice.

His graduate studies began in the Department of Oral Biology of Loyola University School of Dentistry, Chicago College of Dental Surgery, in 1977. Specialty training was in the Department of Orthodontics.

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CHAPTER I

INTRODUCTION

Initially, tissue culture medium methods were designed to study cell anatomy. Later they were used to determine the essential nutrients for growth and development of the cells.

The application of this area of scientific effort became valuable with the observations of the effect of drugs and other materials on the living cells in vitro. This was followed by the wide use of natural and synthetic media stimulating and supporting cell proliferation in vitro but rarely used for research in vivo. Although, there were some studies which determined that the tissue culture medium has stimulating effects on growth and on DNA synthesis in vivo.

There is no reported work on the effect of local administration of tissue culture medium upon cartilaginous growth of the mandibular condyle.

In this study I propose to investigate cell proliferation of the perichondrium in young rabbits as measured by tritiated thymidine labeled cells (in vivo).

CHAPTER II

REVIEW OF LITERATURE

COMPOSITION OF THE CONDYLE AND ITS IMPORTANCE

Many authors, such as Sicher and Weinmann, (1970) have strongly supported their contention that the condyle is the major growth center of the mandible and is endowed with an intrinsic genetic potential.

Koski (1968) and Moss (1970) stated that the condyle is not the principal growth factor for the mandible. The explanation has been that proliferation and differentiation of hyaline cartilage, and its replacement by bone in the deepest layer is quite similar to changes that occur at the epiphyseal plate and in the articular cartilage of the long bone.

Sicher and Duburul (1970) stated the fact that proliferation of connective tissue and interstitial growth of the septal cartilage are the chief factors of growth in the upper facial skeleton but that appositional growth of cartilage is the chief factor of growth of the mandible, which explains a certain independence of these two structures. Also, they stated that the condylar cartilage of the mandible is covered by a thick layer of connective tissue, dense in the superficial and loose in the deep layers. Growth of the condylar cartilage contributes to increase the mandibular ramus height, to increase the over-all length of the mandible, and to increase the intercondylar distance.

Graber (1976) reported in a discussion on condylar growth that endochondral growth does occur during the accomplishment of the full morphogenetic pattern of the mandible. However, because the hyaline cartilage of the condyle is covered by a dense and thick fibrous connective tissue layer, it not only is increased by interstitial growth, as the long bones of the body, but also is able to increase in thickness by appositional growth from perichondrium beneath the connective tissue.

Koski (1968) recommended that the term skeletal growth center be used to describe places of endochondral ossification with tissue separation force. Thus, the term growth center should mean a place where growth of the skeleton is occuring for a sufficient length of time to make a real contribution to the increase of the skeleton mass beyond the size of the model tissue existing at the onset of the growth center function.

Koski (1968) further stated growth in the condyle moves the mandibular body forward and downward and thus opens space below the cranial base into which mandibular and maxillary alveolar processes grow and teeth erupt. Therefore the condyle is considered indispensable for the normal growth of the face.

Sicher (1965) and Salzman (1966) also claimed that the growth of the condylar cartilage is responsible for the antero-posterior growth of the mandible. The general feeling seems to be that here we are dealing with the most important growth center of the lower jaw.

Moss (1960) maintained that the governing agent of facial growth

is the so-called functional matrix. Moss describes the cartilage like a latecomer, a secondary cartilage, and not a part of the Meckel's cartilage, that acts as the model for the early development of the mandible. It is not an articular cartilage, nor an epiphyseal growth plate.

Balinski (1960) stated that condylar cartilage does not even form from the same embryonic precursor tissue as the epiphyseal cartilages, a fact which may have something to do with its structure and function. The information available on condylar growth appears very strongly to point toward its subordinate role as a site of growth, not as growth center, in the growth of the mandible.

Enlow (1975) stated that the mandibular condyle is an anatomic part of special interest because it is a major site of growth, having considerable clinical significance, However, the condyle does not regulate the over-all growth of the entire mandible, including all of its many regional parts. The growth of the mandible as a whole is a result of all the different regional forces and functional agents of growth control acting upon it to produce the topographically complex shape that is the aggregate expression of all these localized factors.

The condylar growth mechanism itself is a clear-cut process. Cartilage is present because variable levels of compression occur at its articular contact with the temporal bone of the cranium. An endochondral growth mechanism is required, because the condyle grows in a direction toward its articulation in the face of direct pressure. An intra-membranous type of growth could not operate, because the periosteal mode

of osteogenesis is not pressure-adapted. Endochondral growth occurs only at the articular contact part of the condyle, since this is where pressure exists at levels beyond the tolerance of the bone's soft tissue membrane. The endochondral bone tissue formed in association with the condylar cartilage is laid down only in the medullary portion of the condyle. The enclosing bony cortices are produced by periosteal-endosteal osteogenic activity; these membranes are not subject to the compressive forces of articulation, but rather, are essentially tension-related, because of muscle and connective tissue attachments.

A TISSUE CULTURE NUTRITION

Animal tissue culture is generally considered to have begun in the early 20th century with the pioneer experiments of Harrison in 1906 with observations on the living developing nerve fiber. The first tissue culture medium was designed primarily for studies of the cell and development.

Carrel (1912) reported that trephones, produced by leukocytes present in injured tissues, were growth promoting substances. After his study, substances such as pleural exudates, extracts of placenta, sulfur containing aminoacids, etc. have been reported to be growth promoting substances for injured tissues (in vitro).

Glasstone (1935) conducted a study to determine if dental tissue would differentiate normally when isolated and cultivated in vitro. Glasstone shows in this experiment that isolated dental tissues are self-differentiating. The results were obtained from cultivation of

tooth germs in vitro which, by eliminating all the vascular and nervous connections, produced a more complete isolation than grafting. The experiment also shows that odontoblast differentiate in vitro only when ameloblast are present. Whole or partial tooth germs cultivated in vitro show remarkable power of physiological differentiation. The dentin papilla developed normally forming odontoblast which deposited normal tubular dentin.

Dentin formation could take place in the absence of the enamel epithelium provided that odontoblasts were present in the explant. Glasstone also stated that one of the functions of the organ enamel is to determine the gross morphological structure of the tooth.

Vogeelar and Erlichmann (1936) reported the significance of the aminoacids for the growth in vitro of the human fibroblast. Using a medium of equal parts of plasma, calcium, ringer, feeding solution, and 2.10% glycine solution, they cultivated thyroid specimens from 13 persons, varying in age from 14 to 48 years. It was concluded that the toxic or beneficial influence of the aminoacids is not primarily dependent upon the concentration, but mainly upon the chemical composition of the medium of which they are forming part.

Teir and Kiljunen (1951) reported that aqueous extract of skin, liver, and spleen, stimulate mitotic activity in homologous organs when they are injected into animals of the same species. This led to the hypothesis that growth promoting substances are liberated from tissue extracts which are organ and specific species.

Morgan (1958) (a study in vitro) stated that the early culture methods were designed primarily for studies on cell morphology and were based on the observation that many tissues could be propagated almost indefinitely in media derived from animal body. New methods have been developed and techniques simplified, to permit the handling of large numbers of replicate tissue cultures. In many instances, these new methods have been devised through the application of long established bacteriological techniques:

A) The development of pure cell strains through single cell isolations and the serial propagations of such strains from cell suspensions are primarily of bacteriological origin.

B) The propagation of monolayer tissue cultures and the formation of plaques in such cultures by animal viruses are analogous to the bacteriophage-bacterial cell system.

These techniques make possible the application to animal virus studies of all the quantitative methods evolved for bacteriophage works.

Modern tissue cultures provide a method through which the morphology, biochemistry, metabolism, survival, growth of individual mammalian cell and progeny of these cells or mixed cell populations may be studied simultaneously under precisely controlled conditions.

Morgan concluded with the concept that present tissue culture media are inadequate to support the growth of small inoculi, unless

the media have previously been altered by the metabolic activity of large, rapidly growing cultures.

IN VIVO GROWTH STIMULATION WITH TISSUE CULTURE MEDIUM (T199)

Toto, Black and Sawinski reported (1968) in a study in vivo, on growth stimulation with tissue culture medium in albino mice. Their conclusion was that tissue culture media (T199), stimulate the proliferation of loose connective tissue in the mouse skin. The cells of the loose, subcutaneous tissue are competent in as much as they can synthetize DNA and differentiate along several lines. Such cells, when placed in tissue culture medium, are capable of proliferation in vitro. They declared that this is not unexpected, that such cells would be stimulated to grow when tissue culture medium was added in vivo.

Toto concluded that, by applying tissue culture medium directly into the tissue, deamination of aminoacids and storage of carbohydrates by the liver was avoided, thus keeping an enriched environment for the competent cells. Also, they declared, it is entirely probable that enriched substrate alone could have this growth stimulating effect, as it is known to occur in the in vitro system free of hemostatic regulation.

Bayardo (1977) reported an experiment on cell density and labeling index of the periosteum in albino rats. Bayardo injected tissue culture medium into the periosteal surface of the interparietal suture, (in vivo); one cubic centimeter of T199 was used for a period of 14 days. He found an increase in the number of cells of the experimental group

as compared to their control. Bayardo concluded that the increased number of cells in the suture is evidence of increased sutural growth and it is suggested that the sutural growth may be modified by enhancement with local administration of enriched medium.

Garcia (1977) also reported the effect of tissue culture medium T199 on the parietal suture in albino mice; he reported a significant difference in the comparison between the experimental group with the control group. He found an increased labeling of cells, using tritiated thymidine label.

Vargas (1978) in a similar study varied the concentrations of tissue culture medium T199, 3%, 5%, 10% and 20%, injected into the periosteal surface of the interparietal suture in rats. He reported that the cell density analysis of the interparietal area showed a high statistically significant increase in the number of cells of the experimental group as compared to their control.

The results in this study indicate that the artificial medium T199 has a stimulating growth effect on DNA synthesis in vivo when it is used in concentrations of 3%. When compared with the 5% T199 there was not a statistically significant increase in the frequency of labeled cells. Moreover, the 10% and 20% concentrations of T199 showed necrosis in the skin and the interparietal area.

INVESTIGATIONS WITH TRITIATED THYMIDINE

Thymidine- H^3 is used as a tool for investigations of the renewal of cell populations. Leblond and Messier stated that it is known that

mitosis is preceded by a synthesis of a deoxyribonucleic acid. Radioactive DNA precursor when administered at cell DNA synthesis enables the nucleus to become radioactive and may be recognized by autoradiography, even before mitosis takes place. Such nuclei retain the label during the actual mitosis and pass it on to the daughter cells.

In the past, phosphate-P^{32} and adenine- C^{14}, have been used to label newly formed DNA and investigate cell populations. While a useful survey of the distribution of DNA was achieved with labeled adenine, the use of this substance, like that phosphate- P^{32}, raises serious problems. The doses required for adequate autoradiography are high enough to cause radiation damage. The detection of labeled DNA requires the extraction of other labeled compounds from the tissue sections, a difficult and often incomplete procedure. Finally, the autoradiography resolution is poor with P^{32} and only fair with C^{14}.

The tritiated thymidine was introduced by the Brookhaven group in 1958. This substance makes it possible to overcome the difficulties encountered with the other DNA precursors. The danger of radiation damage seems to be reduced with tritium, and, it is possible to use doses allowing cells to be traced over periods of months in the body without apparent sign of damage to these or other cells. Furthermore, the sections need not to be subjected to chemical extractions, since DNA is the only labeled substance present in significant amount in the sections following thymidine-H^3 administration; and, indeed, DNA treatments eliminate all autoradiography reactions. Finally, due to the

low beta-ray energy of tritium, the photographic grains produced by tritium containing structures are found within 2μ or 3μ - most of them within 1μ - from the source, as measured within NTB-$_3$ emulsion. It may be concluded that thymidine-H^3 is an adequate tool for the autoradiographic detection of newly formed DNA and, therefore, may be used to locate the sites of cell formation.

EXAMPLES OF INVESTIGATIONS USING TRITIATED THYMIDINE

Joseph, Toto, and Choukas (1968) reported the proliferative capacity and DNA synthesis of osteoblasts during fracture repair in normal and hypophysectomized rats. The proliferative capacity of the periosteum of normal and hypophysectomized rats rapidly rises to a peak within 24 hours following fracture of the fibula. After this, the proliferative capacity decreases, as indicated by the number of cells in DNA synthesis. The catabolism of tritiated thymidine labeled cells results in the liberation of thymidine label, which can be reutilized by tissue cells preparing for duplication. This accounts for labeling for some days after the initial administration of tritiated thymidine.

Addison (1942) stated that subcutaneous injections of tritiated thymidine can be given most easily into the abdominal wall; with the rabbit held in the left hand, the needle is inserted with a quick thrusting motion. If it is desired that the injection be intraperitoneal, the needle is inserted in the Poupart's ligament about two

inches deep.

Sinclair (1976) made an investigation to determine the short term histological change of the facial skeletal complex resulting from rapid palatal expansion by orthodontic appliance in the rhesus monkey. The changes in the sutural area were determined by the histological examination of tissues, labeling index using tritiated thymidine, and cell density. The histological evaluation of the sutural tissue in the experimental animal revealed that there was a decrease in cell density, as the sutural connective tissue was abnormally wide, and the fibers were disoriented.

In view of the foregoing studies indicating that T199 increased cell proliferation in sutural growth of rats and mice, the question concerning the effect on condylar perichondrium cell growth naturally is raised. This study was designed to study the effect of tissue culture medium T199 locally deposited into the capsule of the temperomandibular joint in rabbits.

INVESTIGATION IN RABBITS

Sarnat (1965) reported that the mandible is the largest bone of the facial region, composed of two portions united anteriorly by the symphysis. Each half comprises a horizontal portion, the body of the mandible, and a posterior and vertical portion of the ramus. The latter serves for the insertion of the muscles of mastication and for articulation with the skull. The mandibular ramus forms a broad plate. The lateral surface of which is occupied by the masseter muscle, while

the medial surface forms an area of insertion for the pterygoid muscle. The surface of the ramus is greatly increased in its postero-ventral portion through the expansion of the bone to form the angle. The elongated articular surface is at the end of a vertical or slightly oblique condylar process. Just inferior to this, on the anterior border of the ramus, is the coronoid process. The sigmoid notch is between these two processes.

Pollock (1962) in a comparative study of the masticatory apparatus of a rodent and lagomorph stated that the mandibular articulation in the rabbit is formed by the articular surface of the squamosal bone and the articular surface of the mandible. A disc is interposed between the two bony articular surfaces and separates the joint into an upper and a lower compartment.

The smooth articular surface of the squamosal bone is situated on the inferior surface of the posterior root of the zygomatic process and is slightly raised from the surrounding bone. It faces backward and downward. The mediolateral dimension of the articular surface is approximately twice that of the anteroposterior dimension. The articular surface is oval with an indented posterior border. The articular surface is convex in its anteroposterior dimension but strongly concave mediolaterally. This concavity makes the articular surface appear almost tent-shaped. A depression is seen above the articular surface on the posterior surface of the root of the zygomatic process.

The disc is fused to the very thin capsule by loose connective tissue. It attaches superiorly to the border of the articular surface.

The capsule is quite loose between the squamosal bone and the disc and much tighter between the disc and condyle, but still allows some movements of the disc in all directions.

Bhaskar (1953) reported that the condylar portion of the mandible in the rabbit, as in most other mammalian species, is developed from cartilage. This cartilage is derived from the blastema covering the dorsal extension of the growing mandible, and is first seen in the rabbit at the 17 day insemination age. The condylar cartilage forms an important growth center for the mandibular ramus, somewhat similar to the epiphyseal plate in the long bone, but, from its position within the joint, it also provides the articular surface for the growing mandibular condyle.

Blackwood (1966) reported that the cells in the condylar portion differentiate to become chondroblast or condrocytes and thereby gives rise to the third layer, or hypertrophic cartilage zone, in which the cells and intercellular matrix undergo all the changes to endochondral bone replacement of the cartilage. From a purely structural viewpoint it may be accepted that growth is provided by intermediate and hypertrophic cell zones of the cartilage, and that the articular zone serves as an articular covering for the cartilage.

CHAPTER III

MATERIAL AND METHODS

Six young New Zealand rabbits weighing between one to two kilos, were maintained on rabbit chow and water ad libitum to furnish their dietary requirements (Table 1). Using a 5 cubic centimeters leur lock syringe, three cubic centimeters of air was subcutaneously injected over the capsule of the temporomandibular joint of right and left sides in the six rabbits to create a pouch.

After 24 hours, the control side (right) air pouch was injected with 1 cubic centimeter of buffered physiologic saline solution (0.85%). This procedure was repeated on every third day for 14 days (Table 2). The experimental side (left) was similarly treated as the control group but was administered 1% of an artificial, isotonic tissue culture medium T199 (Table 3), in a concentration of 11.1 grams/liter. The preparation of air pouches and the injections of saline solution or tissue culture medium T199 were made with great care in order to minimize any injurious effect of such administration.

Approximately 8 hours after the last injection, the animals were weighed, and 1μ Ci of tritiated thymidine* (Sp. act. 1.8 Ci/mM) per

*New England Nuclear, Boston, Mass.

TABLE I

DIETARY REQUIREMENTS OF THE RABBITS

			Daily intake
Calcium	0.5-0.6%		40-50 mg.
Phophorus	0.5-0.6%		34-45 mg.
Potassium			15 mg. male 8 mg. female
Sodium	0.5%		
Chlorine			5 mg.
Copper			0.1 mg.
Iodine			2 micrograms
Magnesium			4 mg./kg.
Manganese			0.5 mg.
Zinc			40 micrograms
Protein	25-30% aminoacids	Lysine 1% Tryptophane 0.2% Histidine 0.4% Phenylanine 0.7% Leucine .9% Isolucine .5% Threonine .6% Methionine .6% Valine .7% Arginine .2%	
Fatty Acids (Methyl linolate)			25 mg.

TABLE I continued

Vit. A	4 Micrograms
Carotene	15-20 micrograms per/kg.
Thiamine	10 micrograms
Riboflamin	10 micrograms
Pyridoxine	40 micrograms
Vit. E (Alpha tocopherol)	1 mg.

TABLE 2

SCHEDULE OF FREQUENCY OF ADMINISTRATION
OF SALINE SOLUTION
AND TISSUE CULTURE MEDIUM (T199) ON RABBITS

DATE	CONTROL	EXPERIMENTAL
May 22 1978	3 cc. Air	3 cc. Air
May 23 1978	1 cc. Saline sol.	1 cc. T199
May 25 1978	1 cc. Saline sol.	1 cc. T199
May 27 1978	1 cc. Saline sol.	1 cc. T199
May 29 1978	1 cc. Saline sol.	1 cc. T199
May 31 1978	1 cc. Saline sol.	1 cc. T199
June 2 1978	1 cc. Saline sol.	1 cc. T199
June 4 1978	1 cc. Saline sol.	1 cc. T199

TABLE 3

COMPOSITION OF THE TISSUE CULTURE MEDIUM T199

COMPONENT	mg/L
INORGANIC SALTS	
$CaCl_2$(anhyd)	140.00
$Fe(NO_3)_3.9H_2O$	0.72
KCl	400.00
KH_2PO_4	60.00
$MgSO_4.7H_2O$ (anhyd)	97.72
NaCl	8000.00
$Na_2HPO_4.7H_2O$(anhyd)	47.70
OTHER COMPONENTS	
Adenin sulfate	10.00
Alpha Tocopherol	0.01
Adenylic Acid	.20
Chloesterol	.20
Adenosinetriphophage	1.00
Deoxiribose	0.50
Glucose	1000.00
Glutathione	0.50
Guanine	0.30
Hypoxantine Na salt	0.35
Phenol red	20.00
Ribose	0.35
Sodium acetate	50.00
Thymine	0.30
Tween 80 (TM)	20.00
Uracil	0.30
Xanthine Na salt	0.34

COMPONENT	
AMINOACIDS	
DL-Alpha Alanine	50.000
L-Arginine	70.000
DL-Aspartic acid	60.000
L-Cysteine HCL-H_2O	0.110
L-Cysteine 2HCl	26.000
L-Glutamic acid	150.000
L-Glutamine	100.000
Glycine	50.000
l-Histidine	21.880
L-Hidroxyproline	10.000
DL-Isoluecine	40.000
DL-Leucine	120.000
L-Lycine HCl	70.000
DL-Methionine	30.000
DL-Phenylalanine	50.000
L-Proline	40.000
DL-Serine	50.000
DL-Thereonine	60.000
DL-Tryptophan	20.000
L- Tyrocine	57.880
DL-Valine	50.000
Vitamins.	
Vitamin A (Aceteate)	0.140
Ascorbic acid	0.050
d-biotin	0.010
Calciferol	0.100
Ca Pantothenate	0.010
Chlorine Chlride	0.500
Folic acid	0.010
i-Inositol	0.050
Menadione	0.010
Niacin	0.025
Niacinamide	0.025
Para-aminobenzoic acid	0.050
Pyridoxal HCl	0.025
Riboflavin	0.010

gram of body weight was administered, intraperitoneally, to each one of the rabbits. One hour post-injection, the animals were sacrificed using an overdose of chloroform. The condyles were removed by sharp dissection, cut into blocks 1 X 1 centimeters and fixed in cold neutral formalin for 8 hours. Following washing, dehydration in ascending alcohol, and clearing in xylene, they were embedded in paraffin. Slides were prepared for microscopic examination by cutting sections of 6μ thick.

Autoradiograms of the sections were prepared with NTB_3 liquid photographic emulsion,* in a photographic darkroom. The slides were sealed in light-tight microscopic slide boxes and placed in the freezer. After 5 weeks exposure at 0° Centigrade, the sections were developed, washed, fixed, and stained with Nuclear fast red and indigo carmine counterstain. The slides were examined under a binocular microscope with an eyepiece reticular of $100\mu^2$ at 100 X. The nuclei counts were determined utilizing a manual blood cell counter. The nuclei of the perichondrium of randomly selected sections were counted in both experimental and control groups.

A total of $1000\mu^2$ of perichondrium were made for each specimen by ten randomly counted fields $100\mu^2$. The mean and standard deviation of the cell density per $100\mu^2$ were calculated. The "t" test was applied

*NTB_3 Kodak, Rochester, New York

to the samples for signigicant differences between the means.

LABELED CELL COUNTS

The tritiated thymidine labeled cells of the perichondrium in $1000\mu^2$ area of the previously ten randomly selected fields were counted, in both control and experimental groups. Using the $100\mu^2$ recticular, as employed for cell density counts of the perichondrium for each specimen, the number of labeled cells per $100\mu^2$ was determined.

The mean and standard deviation of labeled cells per $100\mu^2$ were calculated. The "t" test was applied to such data for determining significant differences between the means of the control and experimental groups.

CHAPTER IV

RESULTS

OBSERVATION OF THE RABBITS DURING EXPERIMENTAL PERIOD

The rabbits treated by the injection of T199 and saline solution into the capsule of the temporomandibular joint appeared to behave normally during the experimental period of 14 days. They consumed rabbit chow and drank water liberally. There was not any apparent lesion at the skin sites of injection, moreover, there was no observable disturbance or affection in chewing as seen by inspection. The examination of the perichondrium, as it appeared grossly at the time of excision of the mandible, showed only apparently normal smooth curved surface.

MICROSCOPIC EXAMINATION OF THE CONDYLE

Microscopic observation of the perichondrium of the condyle of the rabbit, and cartilage showed a normal pattern of proliferation, differentiation of chondrocytes, calcification and removal by osteoclast at the bony spongiosa aspect. Such observations suggest that injections either by saline, or 1% T199 tissue culture medium were well tolerated by the condyle.

CELL DENSITY

The perichondrium of the mandibular condyle showed an increase in

cell density in the T199 treated organ as compared to that treated with saline alone. The mean cell density in the experimental perichondrium was $116.03/100^2$ while that in the control was $112.80/100^2$. The finding was significant (Table 4).

LABELING INDEX WITH TRITIATED THYMIDINE

The frequency of labeled nuclei in the perichondrium of the T199 treated capsule of the mandibular condyle in the rabbits was greater than seen in the saline treated controls. The labeling index of the cells in the perichondrium of the experimental showed a mean of $6.03/100^2$ cells as compared to 4.03 of the saline treated specimens. Thus, was highly signigicant (Table 4). Tissue culture medium T199 injections into the capsule appears to cause an increase in the rate of growth, as indicated in Table 4, by the increase in cell density and tritiated thymidine labeling indices.

HISTOLOGIC OBSERVATIONS

The articular surfaces of the mandibular condyle are arcuate, with their axes placed in the same direction as those of the articular tubercles on the temporal bone. They are supported by a fibrous capsule fixed to the temporal bone and the condyle. The articular capsule is a fibrous sac strengthened laterally by the temporomandibular ligament. The inner aspect of the capsule is lined by a synovial membrane. The Synovial membrane lines the joint capsule, being especially well developed behind the disc, but does not extend over the surface of the disc, the

TABLE 4

EFFECT OF TISSUE CULTURE MEDIUM T199 ON THE CELL DENSITY AND LABELING INDICES IN THE PERICHONDRIUM OF THE MANDIBULAR CONDYLE IN THE RABBIT

	CELL DENSITY No. cells/100μ^2	LABEL INDEX No. cells/100μ^2 Labeled with Tritiated thymidine
CONTROL	M= 112.8	4.03
	SD= 4.9	1.93
EXPERIMENTAL	M= 116.03	6.03
	SD= 5.72	2.69
"t" value	2.95	7.03
.01	1.66	2.61

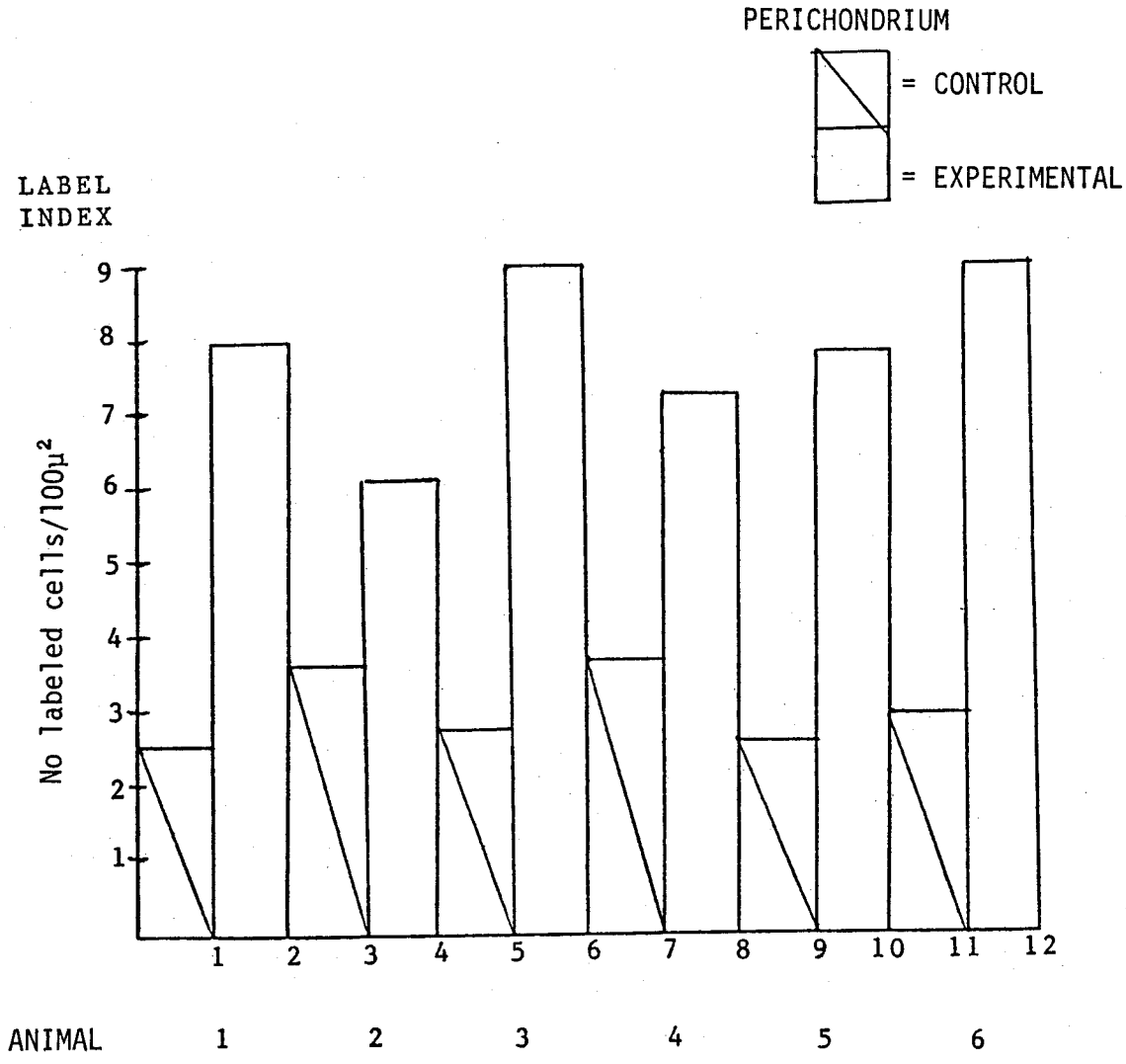

Figure 1 Comparison of the labelling indices of the perichondrium of the mandibular condyles in rabbits treated with T199 and saline.

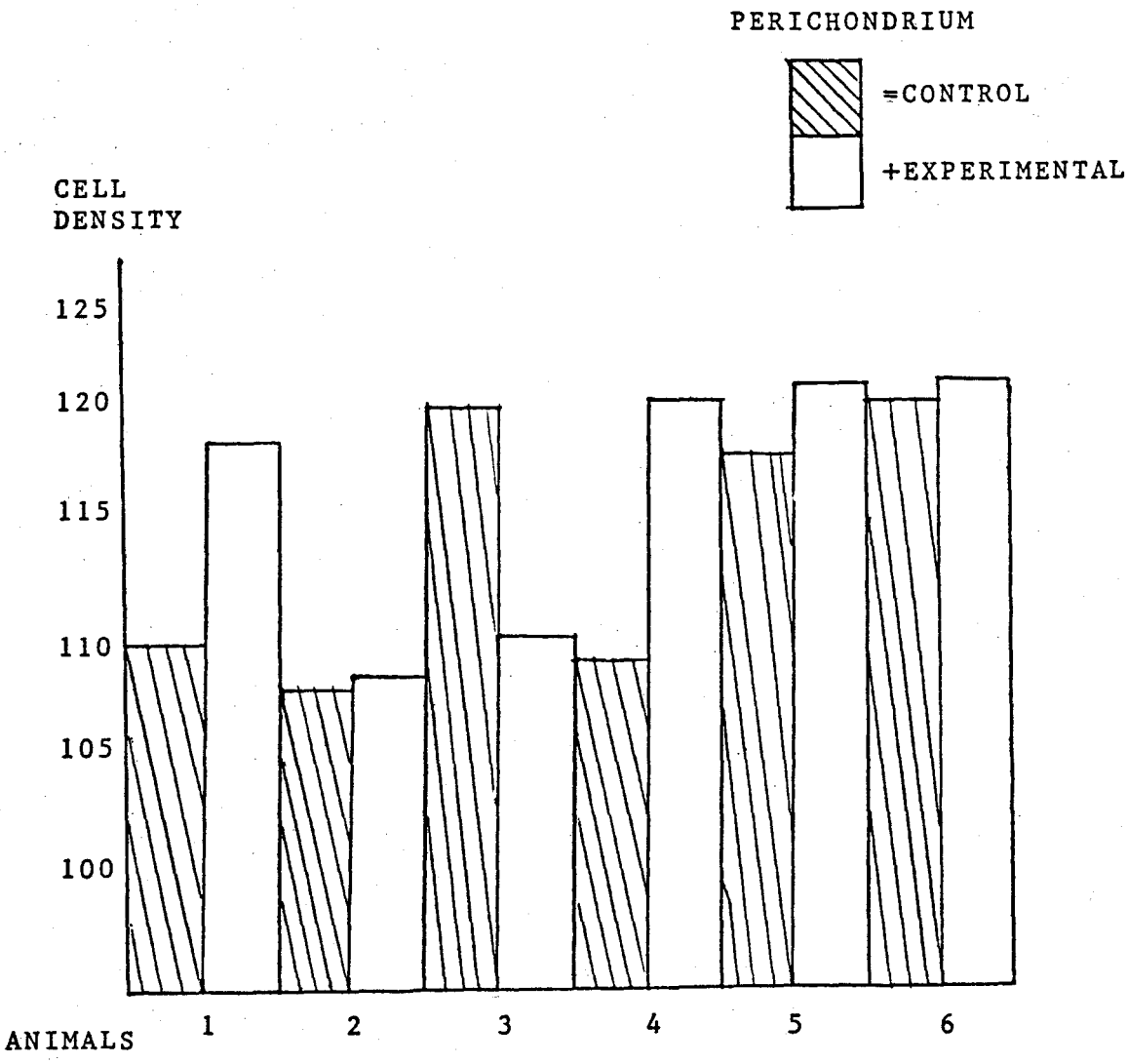

Figure 2 Comparison of the cell densities of the mandibular condyles in rabbits treated with T199 and saline.

articular tubercle, or the condyle. Articular fibrous tissue covers the condyle as well as the articular tubercle and contains variable numbers of chondrocytes.

A layer of hyaline cartilage lies underneath the fibrous covering of the condyle. This cartilaginous plate grows by apposition from the deepest layers of the covering connective tissue. At the same time its deep surface is replaced by bone. The condyle of the mandible is composed of cancellous trabeculae grouped in such a way that they radiate from the neck of the mandible and reach the cortex at right angles, thus giving maximal strength to the condyle.

CHAPTER V

DISCUSSION

The objective of early experiments in synthetic media for cell cultures was to devise an adequate medium to support unlimited cell survival and multiplication. At the present time, the composition of the synthetic media most used include as many nutritional factors as possible already shown to be necessary for man, animals, and bacteria.

The artificial medium T199 contains the essential elements required for cell growth and proliferation; therefore, the injections of this substrate into the preformed air pouches reproduced, to a great extent, its *in vitro* and *in vivo* qualities reported by previous experiments of Morgan in 1958 and Toto in 1968.

As mitosis is preceded by synthesis of DNA; and as thymidine is a specific precursor of DNA, the incorporation of tritiated thymidine into the DNA molecule serves to demonstrate the extent of DNA synthesis and mitosis occuring in the perichondrium of the condyle.

In regard to the histological morphology of the condyle, three layers were observed: Fibrous covering, with a thick layer, its superficial layers consist of a network of strong collagenous fibers, some chondrocytes were present. The deepest layer of the fibrocartilage is rich in chondrocytes indicative of growing hyaline cartilage. In

this zone the appositional growth of the hyaline cartilage of the condyle takes place during the period of growth; also, this was stated by Orban and Pollock (1962).

In this work, the labeling index represents the quantity of cells that are preparing for mitosis during a one hour period established after the injection of radioactive thymidine. The high significant difference found in the index represents the mitotic activity of the cells of the perichondrium of the condyle eight hours after the last injection of saline solution or tissue culture medium T199. The increased labeling index in the experimental group suggests that the artificial medium used in this study stimulates cell proliferation under specified conditions.

The slight difference in cell density between the control and experimental groups indicates that, either the cells enlarge due to differentiation, or, leave the proliferative compartment and differentiate to chondrocytes. The number of cells per unit area would be reduced in either case. The significant increase in the labeling index in the experiment represents evidence of increased mitotic activity as a function of the tissue culture medium T199 administered during the two week experimental period. The increased number of cells in the area of the perichondrium of the condyle is evidence to suggest increased growth.

The probable mechanism of action of the tissue culture medium seems to be related to the nutrient enrichment of the local environment

of the competent cells. This mechanism probably is similarly related to the systemic effect of the growth promoting substances used in intravenous feeding hyperalimentation. Thus, it is suggested that condylar growth may be modified by enhancement by local administration of enriched medium. However, it is difficult to define, in this study, which is the specific factor that propitiates these changes. Furthermore, this study supports the reported findings for the in vivo local growth promoting activity of tissue culture medium T199 (Toto, 1968; Bayardo, 1977; Garcia, 1977; Vargas, 1978).

SUMMARY AND CONCLUSIONS

The mandibular condyles of six young rabbits were divided in two sides; experimental condyle left and control condyle right to make a total of 12 condyles. Three cubic centimeters of air was subcutaneously injected into the temporo-mandibular junction to create a pouch.

After 24 hours, one cubic centimeter of tissue culture medium or saline solution was injected into the pouch of the experimental and control groups, respectively. The same procedure was repeated every two days for a total period of two weeks.

Eight hours after the last injection, the animals were intraperitoneally injected with tritiated thymidine at the rate of one microcurie per gram of body weight. One hour later all the animals were sacrificed.

Histological sections of the condyle area were prepared for autoradiograms. Autoradiographic analysis of the perichondrium of the condyles showed a high increase in labeled cells of animals receiving tissue culture medium T199 as compared to the saline control animals.

Cell density analysis of the perichondrium of the condyle showed a slight statistically significant increase in the number of cells of the experimental group compared to their control.

The results of this study indicate that the artificial medium

has stimulating effect on the growth of the perichondrium of the condyle of the rabbits.

The increased number of cells in the perichondrium of the condyle is evidence of increased condylar growth, and it is strongly suggested that the condylar growth may be modified by enhancement with the local administration of enriched tissue culture medium.

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APPROVAL SHEET

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

1-12-1981
Date

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