1983

The Effect of Tissue Culture Medium (T199) on the Local Condylar Growth of the Rabbit in Vivo

Nouraddin Ali Nuseir
Loyola University Chicago

3/

THE EFFECT OF TISSUE CULTURE MEDIUM (T199) ON THE LOCAL CONDYLAR GROWTH OF THE RABBIT IN VIVO

by

Nouraddin Ali Nuseir, D.D.S.

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF LOYOLA UNIVERSITY OF CHICAGO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

December

1983

CONTENTS

ACKNOWLEDGEMENT

I would like to express my sincere appreciation and gratitude to my advisor Dr. Patrick D. Toto for his constant cooperation, supervision and patience.

I'm very grateful to Dr. Michael Kiely and Dr. Kirk Hoerman for their continuous advices and guidance. Special thanks to Mr. Robert Martinez, a histology technician, for his assistance and cooperation.

Also, I wish to thank my society who gave me this opportunity to continue my graduate studies.

In addition, I extend my special gratitude and love to my wife and my family for their support and encouragement.

"My Lord! Increase me in Knowledge."

CURICULUM VITA

Nouraddin Ali Nuseir was born in Zawia, Libya, on June 26, 1950. His secondary education was received at Zawia Secondary School from 1964-1970, while his college education was obtained at School of Science, Cairo University in Egypt from 1970-1971.

In September 1971, he began dental studies at Cairo Univ. Dental Faculty, where he received his DDS in May 1975.

In August 1975, he started his dental practice in Libya and continued until August 1980. During this time, he participated with his community in many social activities such as public health and red crescent services. In November 1975, he was elected as a Health Services Chairman in Zawia Province, in Libya. In March 1976, he was again elected as a Chairman of Libyan Red Crescent (Cross) in the same area. In May 1977, he was elected as a General Secretary Assistant of the Libyan Medical Profession Associations. Also, as a health servant, he attended many national and international conferences, meetings, and congresses.

In September 1980, he studied English in Denver, Colorado and in Chicago, Illinois. In September 1981, he started a two year graduate program at Loyola University, School of Dentistry, Chicago, leading to a Master of Science in Oral Biology and a postgraduate certificate in Pedodontics. In September 1983, he started a Ph.D. program in Oral Biology at Northwestern University, Chicago, Illinois.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Nutritive media, which have been used for implantation of animal cells to study their anatomy in the tissue cultures, have been derived first from animal blood sera. Later, nutritive media comprised of chemically-defined substances have been devised and used to determine the essential nutrients required for growth and development of cells.

The application of tissue cultures became very valuable for the study of effects of chemicals such as drugs on the living cells in vitro and to promote and support the stimulation of cells proliferation and differentiation in vitro but rarely in vivo.

The purpose of this study was to investigate the cell proliferation and differentiation of perichondrium (in vivo) in young rabbits, as modified by periarticular administration of the tissue culture medium T199. Tritiated thymidine labelling of the proliferating populaton was used to determine the labelling index as a measure of growth. Furthermore, as the cells differentiated, the labelled cells were detected and quantitated in the differentiated compartment of cartilage cells. Also, the vertical thickness and the greatest anteroposterior measurements of the condyle were made. This study suggests the parenteral, local administration of 1% T199 promotes growth in vivo which is supported

by the findings that the labeling indices, cell densities and the cartilage thickness increases in the condyles of six week old growing rabbits. While the data were not completely statistically significant, they strongly suggest that some increase in concentration or frequency of the local administration of T199 may produce positive effects on condylar cartilage growth.

REVIEW OF THE RELATED LITERATURE

Growth activities of the secondary cartilage of the mandibular condyle such as the rate of proliferation, differentiation and extracellular matrix production have been studied using autoradiography following the administration of the tritiated thymidine (Blackwood., 66; Folk and Stallard, 67; Oberg et al., 67; Kanouse et al., 69; and Joondeph, 72.) Quantitative data estimating the thickness of the various demarcated tissue layers have been published by Stockli & Willert, 71, and Carlson et al., 78.

Many studies to determine the mechanism and the cartilage proliferation site in the condyle have been conducted (Folk & Stallard, 67; Oberg 68; Moss, 68; Kanouse, 69; Joondeph, 72; and Carlson, 78). According to Moss, 68, the mandibular condyle is not a center of growth, rather it is an independent mandibular skeletal unit. The condylar cartilage is not primarily responsible for growth of any other mandibular skeletal unit except the condylar process itself. It is emphasized by Moss that normally the condylar cartilages are not primary growth sites in any way responsible for the mandibular growth as a whole, but rather act as sites for secondary and compensatory growth of the condylar growth alone, while other authors such as Sicher, 65; Blackwood, 66; and Enlow, 75; consider the mandibular condyle as a major site of the mandibular growth. However, the

condylar growth mechanism itself is a clear-cut process.

CONDYLAR DEVELOPMENT, ANATOMY AND HISTOLOGY

Developmentally, the condylar portion of the mandible in the rabbit, as in most other mammalian species, is developed in cartilage which is derived from the blastema covering the dorsal extension of the growing mandible (Bhaskar, 53). This differs from other mandibular skeletal units which originate by intramembranous bone formation.

Anatomically, the temporomandibular joint is highly specialized and distinguishes itself from other synovial joints. It is unique in its composition in that the articular surfaces are covered by avascular fibrous tissue rather than hyaline cartilage tissue found in other joints. The articular surfaces of the mandibular condyles are different from the other synovial joints, in that they contain a structural perichondrium which functions like cartilage in the process of the endochnodral bone formation.

The condylar process in the rabbit is vertical or slightly oblique and covered with an elongated articular surface at its end (Sarnat and Wexler, 1966.) Pollock, 1962, in his study comparing the masticatory apparatus of the rodent and lagomorph, stated that the mandibular articulaton in the rabbit is formed by the articular surface of the squamosal bone and the articular surface of the

mandible which are separated by a disc forming the upper and the lower compartments of the joint. The mediolateral dimension of the articular surface is oval with an indented posterior border, and it is convex in its anteroposterior dimension, but strongly concave mediolaterally appearing almost tent-shaped (Fig 2). The disc is superiorly attached to the articular surface by a capsular ligament. The capsule is quite loose between the squamosal bone and the disc and much tighter between the disc and the condyle but still allows some movement of the disc in all directions.

Histologically, Blackwood divided the mandibular condylar cartilage into three zones; a superficial articular layer; an intermediate zone; and a hypertrophic zone. The intermediate zone is regarded as the main progenitor or growth layer of the cartilage, and mitosis is observed much more within this zone than the other zones of the cartilage. The cells of this intermediate zone differentiate into chondroblasts or chondrocytes and give rise to the layer of hypertrophic zone in which the cells and the intercellular matrix undergo all the changes advancing to the endochondral bone replacement of the cartilage. The intermediate and hypertrophic cell zones of the cartilage provide the growth, while the articular zone serves as an articular covering for the cartilage.

Luder et al., 82, for the purpose of designing a morphometrical model system using PAS-haemotoxylin staining

distinguished four layers within the cartilagenous covering of the condyle in the monkeys, and also defined a particular zone of underlying bone. On the basis of structural criteria, they defined the layers as; fibrous layer; intermediate layer; transitional layer; hypertrophic layer; and the zone of cartilage resorption and bone formation.

Kanouse et al., 1969, investigated the condylar growth in primates using tritiated thymidine. They observed that the proliferative zone is the primary condylar growth site, and they concluded that two separate sites of cellular division were present within the condyles; the proliferative layer of undifferentiated mesenchymal cells which contributing to both condylar growth and cellular renewal, and the articular zone which related to the cell renewal within this zone, independant of growth activity.

Silbermann and Frommer, 1972, studied the activity of the chondrocytes in the mandibular condyle in primates, they found that the cartilagenous tissue of the mandibular condyle maintains the morphologic and metabolic characteristics of an embryonic type of tissue. The cartilage cells in the condyle lack the specific arrangement and cellular homogenicity characteristic of the more differential endochondral growth sites. They noticed that the differentiated chondrocytes appeared to differentiate further into specialized cells, possibly osteoprogenitor cells, which support the concept that calcification in the condylar cartilage is not

necessarily accompanied by degeneration and death of the chondrocytes.

TISSUE CULTURE NUTRITION AND IN VIVO GROWTH STUDIES

The nutritive media, which have been used for the implantation of the animal cells in the tissue cultures, have been derived first from blood plasma, blood serum, body exudates and various tissue extracts. Later attempts have been made to devise nutritive media compromised of chemically defined substances. Morgan, 1950, established an optimal concentration of amino acids, vitamins, nucleic acids and various accessory growth factors for the maintainance of the cell life in vitro and a completely synthetic solution has been devised and named T199. The tissue culture media have been used by many investigators to support the growth of animal cells both in vitro and in vivo.

Toto et al., 1968, concluded that the findings in their experiment, using the culture media in vivo to stimulate the growth, suggest that conditions in living systems which can make available to cells rapidly usable raw materials for growth may stimulate and/or support the growth. They found in an autoradiographic study a significantly greater number of labeled cells and significantly greater amount of radioactivity in the walls of subcutaneous pouches containing T199, which suggest that the tissue culture

medium may support and probably stimulate growth.

Bayardo, 1977, reported that the artificial medium T199 has a stimulating effect on the growth of the sagittal sutures on the albino rats. He found that the number of the cells in the sutures which received tissue culture medium T199 as compared to the saline control animals is higher which strongly suggests that the sutural growth may be modified by enhancement with local administration of enriched medium.

Haupers, 1978, conducted a study using tissue culture medium T199 in an alloplastic implantation in osseous defects in primates. Histologically, he observed more bone generation and osteoblastic activity in the medium T199 specimens than was seen in the control specimens.

Eastman, 1980, in his study alloplastic implants in primates using the calscorbate and the culture medium T199 concluded that both the calscorbate and the culture medium were accepted by the host site and a favorable osteogenesis had occured. The grafts may have acted by creating an environment rich in nutrients mainly supporting the growth of adjacent bone and the osteogenic cells by inhibiting the size of the blood clot and/or by acting as a "space occupier" allowing for great numbers of undifferentiated mesenchymal cells and host induction of osteogenesis.

In the experimental animals, functionally and histologically successful transplants of small whole joints

appear to be dependent on providing sufficient nutrition to the transplanted articular cartilage, under experimental conditions designed to prevent the repair process in the underlying metaphyseal and subchondral bone to reach the articular cartilage and on certain mechanical factors. With the concept of providing nutrition to the articular cartilage, clinical autotransplantations of a femoral head and an upper end of the tibia in man were carried out. The metaphysis was replaced with methylmethacrylate and the cartilage was kept viable in the artificial tissue culture media during the process of the bone filling with the methylmethacrylate. This demonstrates that the tissue culture media can be used to maintain the cells of the articular cartilage viable for extended periods of time (Glimcher, 1973).

MALNUTRITION EFFECTS ON GROWTH AND DEVELOPMENT

The effects of malnutrition on growth and development have been intensively conducted using the experimental animals. The results strongly suggest that malnutrition such as low-grade protein calorie deficiency in lactating rats caused a retardatin in the development of the mandible and long bones in the offspring. Such findings suggest that proteins are essential nutrients required for cartilage growth.

Srivastava et al., 1972, studied the metabolism of

the nucleic acids and proteins and the cellular growth in various organs of female rats subjected to a diet restriction during the period of gestation, as well as the period of growth, and lactation. Generally, they found both body and organ growth were retarded during this restriction of the nucleic acids and the proteins in the diet.

McAnulty et al., 1974, studied the effect of malnutrition and rehabilitation on the growth of rats. They found that the body weight was increasing more rapidly than the normal during the rehabilitation, and that the undernutrition retards the growth rate. This agrees with many other studies in which the investigators have reported that the malnutrition retards the rate of growth in the young animals and children, and the effect on permanency depends upon the timing, duration and severity of the insult.

Kiely, 1977, reported that the effects of magnesium-deficient diet on the rats were a significant decrease of the eruption rate of the teeth as compared to rats fed on standard diet, and the cell count data revealed a marked reduction in the oral tissues.

Pucciarelli, 1982, investigated the growth of the functional components of the rat skull and its alteration by nutritional effect. He stated that the nutrition strikingly influences the normal pattern of the growth and development of all organs. He concluded that nutritional deficiencies seem to affect to different degrees both splanchnocranial

components, and the growth of masticatory components are markedly inhibited by the nutritional stresses. Additionally in 1980, Pucciarelli has observed that under certain experimental conditions a nutritional factor may stimulate the cranial differentiation to an extent greater than that brought about by the genetically controlled factors. He concluded that nutritional factors can modify a taxonomic distance in three different ways: evoking morphological differences among a population of the same racial group; altering differences among racial groups; and modifying the pattern of sexual dimorphism of the populations.

Slavkin et al., 1982, evaluated the requirement of the vascular and/or neurotrophic derived factors on the determination and differentiation of the chondrogenic and osteogenic phenotypes. Early embryonic quail and mouse mandibular processes were cultured using a modified Trowell method in a serumless, chemically-defined medium for 10 days. Quail HH stage 22 and mouse Theiler stage 16 mandibular processes formed cartilage and produced osteoid under these experimental conditions in vitro. The chondrogenic and osteogenic phenotypes were expressed without serum or other exogenous growth promoting influences demonstrating the support of the embryonic growth by the chemically defined media.

The best route for satisfying nutritional requirement is the gastrointestinal tract. However, Dudrik et al.,

1968, investigated the effect of a long term parenteral nutrition on growth, development and positive nitrogen balance. They stated that the most dramatic result of a total intravenous nutrition was the normal growth of a 1.8 kilogram infant with near total small bowel atretia. They claimed that it was the first demonstration that the growth, development, and the positive nitrogen balance can be achieved by long-term total parenteral nutrition in animals and man, using the chemically-defined media.

TRITIATED THYMIDINE INVESTIGATIONS

Tritiated thymidine has been used quantitively and qualitatively in the growth studies because of the thymidine specificity. The thymidine, as a DNA precursor, is labelled by the cells undergoing DNA synthesis prior to the mitotic divisions (Joondeph, 1972). Quantively, by tracing the fate of the labeled cells or by the identification of a precursor of a certain cell type, the tritium is in a stable configuration on the thymidine molecules and is retained within the DNA through the live of the cell. The isotope reutilization can take place only after the death of the previously labeled cell (Hughes, 1959).

3H-thymidine specifically labels the DNA during chromosomal replication and owing to its accurate localization within the cell, it may be detected within the cell

nucleus and in succeeding cell divisions (Blackwood 1966). The rapidly proliferating cell system uptake of tritiated thymidine may be accepted as an index of the proliferative activity of the tissue. The daughter cells arising from the labeled mitoses enter the postmitotic G_1 phase where some recommence the cell cycle while others differentiate to perform the functional activities characteristic to that cell. These cellular processes in G_1 are continuously influenced by environmental factors such as pH and nutritional conditions which are considered to be of a great significance in regulating the incidence of the mitosis and proliferation rate.

Folk and Stallard, 1967, studied the cell behavior in different structures of the rat mandibular joint. Using tritiated thymidine they found that the greater labeling index was in the embryonic zone in which the cells revealed a cell cycle of 100 hours. After the division, about 50% of the cells remained in the progenitor pool to maintain the cell population and the others specialized to chondroblasts. The labeled chondrocytes following the differentiation retained their labeling during their migration within the condylar cartilage, which finally was released into the medullary cavity in 5 - 7 days.

As the tritiated thymidine is injected into an animal, it is incorporated into the DNA in the cells only during the replication of their genetic material during S phase

prior to the mitotic division. Approximately half the radioactivity is distributed to each daughter nucleus and the label will be carried through a number of cell generations because of the metabolic stability of the DNA. The labeled cells are visualized and localized by using autoradiography, and the successsive generation may be observed if the tissue samples are collected in appropriate intervals. This technique has been firmly established as means of analyzing the cellular proliferation, migration and specialization.

Joondph, 1972, investigated the temporomandibular articulations in growing monkeys and found that the thymidine labeled cells were present in the autoradiographs from all studied animals, such cells were not seen in the condylar growth cartilage of the animals sacrificed 1 1/2 hours following the isotope injection. He explained that there was not enough time for the isotope to reach the avascular germinal zone of the growth cartilage. Access to this area after the absorption from the peritoneal cavity into the vascular system requires the diffusion through subsynovial capillary plexus into the synovial fluid and then through the articular tissue into the underlying proliferative zone. Joondeph suggested that an interval slightly longer than 1 1/2 hours is necessary to accomplish labelling within the condylar growth cartilage. Luder and his colleagues, (1982) chose an interval of 3 hours intervening between the administration of the label and the perfusion of the animals be-

cause Luder, in a previous unpublished study, found that his results confirm the results of Joondeph ('72) that no labeled nuclei could be found in the cartilagenous covering up to 1 1/2 hours after the injection.

The reports in the literature cited suggest that growth of cartilage, in part, can be maintained in tissue culture, including whole joint explants. A measure of growth may be secured by tritiated thymidine labelling of chondrocytes, also. This study proposes to measure the effect of tissue culture medium in vivo on cartilage growth in the rabbit mandibular condyle, using tritiated thymidine as a marker.

PROCEDURES

Thirteen New Zealand white rabbits six week old were maintained on rabbit chow supplemented daily with fresh vegetables (carrots, lettuce) and freely available water. A subcutaneous air pouch was prepared over the left and right temporomandibular joint capsule, by injecting 5 cc of air using a 5 cc syringe and 25 gauge needle.

The right and left side air pouches randomly were assigned to experimental and control groups. 5 ml of 1% T199 in phosphate buffered saline (PBS) carefully was injected into the air pouches on the experimental side while 5 ml PBS was injected into the air pouches on contol side of all rabbits, using a 5 cc syringe and a 25 gauge needle. Such injections were repeated daily for seven days. After a 24 hour rest period, the subcutaneous injection was directed into the capsular tissue of the temporomandibular joint, using a 1 cc tuberculin syringe and 25 gauge needle, delivering 100 uCi tritiated thymidine (specific activity 2.0 Ci/mM) (New England Nuclear Boston Mass) to the experimental side and also to the control side of all rabbits.

At this point in the procedure, eight rabbits were randomly selected and sacrificed using ethyl ether inhalation three hours following the injection with tritiated thymidine in order to determine pulse labelling in the perichondrium. The five remaining rabbits were permitted to

remain alive for seven additional days, to permit time for nucleic division to occur in the perichondrium and the migration of the cells from the perichondrium into the differentiative zone of the cartilage formation, before killing. This procedure provided two groups; first, group one for the study of tritiated thymidine pulse labelling of the proliferative population of cells in the perichondrium after seven days treatment with T199; second, group two for the study of a labelled cell population within maturing and cartilage synthesizing cells, after seven days of treatment with T199 plus additional seven days of growth without such treatment.

The condyles were sharply dissected from all of the rabbits and examined grossly for the presence of smooth glistening articular surfaces and fixed in 10% cold neutral buffered formalin. The condyles were removed from formalin after 48 hours, and washed overnight in tap water. The condyles were placed in 1 liter of sodium citrate-formic acid 50:50 solution for decalcification. The condyles periodically were examined by roentgenographs for the presence of radiopacities; and then observed to be completely radiolucent, the condyles were washed in tap water, dehydrated in ascending series of alcohols and cleared in xylene. Embeded in paraffin, the condyles were serially sectioned in the anterior-posterior plane at 6 microns, using an AO-810 microtome. The sections were deparaffinized in xylene, brought to water and placed on glass slides.

In a dark room, the slides were dipped into NTB, (Kodak) liquid emulsion, removed, allowed to drain and placed into plastic microscopic boxes, contained lithium carbonate dehydrate in a thin paper fold. The slide boxes were placed into a refrigerator at 4 C^{o} for six weeks. In a dark room the slides, were removed from the slide boxes, transferred to a glass slide holding tray and the emulsion was photographically developed, washed and fixed. The slides then were stained with safranin and hematoxylin, and mounted with a coverslip using Permount.

DETERMINATION OF LABELLING INDICES AND CELL DENSITIES

The sections were examined with a binocular microscope (AO-10) using a reticular grid in one ocular with an area covering 0.25 mm^2, at 400 magnification. Ten to twelve sections randomly selected from each specimen were examined. Only the nuclei were counted in randomly selected fields of 0.25mm^2 each. This procedure was done first only in the perichondrium in group 1 and secondly in the subperichondrial differentiating layers or cartilage forming chondrocytes only in group 2 where 25 fields were counted per section and each 5 fields were summed together to give an average per 1.25 mm^2 . However, the mean and standard deviation were counted per 0.25 mm^2 . This was done to find as much labeled cells as possible per section in this group.

This method provided for both cell density, the number of cells per 0.25 mm^2, and simulataneously, provided for a labelling index, counting the numbers of the tritiated thymidine labelled nuclei per 0.25 mm^2. A mean and standard deviation were determined, thereby, using such values and by employing the Student "t" test, the significance of the differences of the means were calculated.

In group 1, such calculations include the means of the number of nuclei per 0.25 mm^2 (cell density) and the means of the number of the labeled nuclei per 0.25 mm^2(labelling index) only in the perichondrium of both the experimental and the control condyles of the eight rabbits pulse labeled with tritiated thymidine.

Similarly, the values obtained from the subperichondrial chondrocytes in the cartilage matrix producing cells of the five rabbits, allowed to grow and live for seven additional days (group 2), following the pulse labeling with tritiated thymidine, also provided both labeling index and cell density.

MEASUREMENTS IN THE THICKNESS OF

CARTILAGE OF THE MANDIBULAR CONDYLES

Ten sections each from the experimental and control condyles taken from the eight rabbits in group 1, and twelve

sections of the experimental and control condyles taken from the five rabbits in group 2 were measured for the thickness of cartilage. Two planes of measurements were selected. The vertical thickness was taken from a point at highest crest of the curved condylar surfaces and descending on an imaginary perpendicular line from the point to a second longest imaginary line, coursing anteroposteriorly in mid saggital plane representing the thickest dimension through the region of the junction of the calcified cartilage with newly formed cancellous bone of the mandible (Fig. 1). All such measurements were made with aid of 2 mm linear calibrated slide (American Optical), divided into millimeter units, and examined with a binocular microscope (AO-10) at 400 and 100 magnification respectively. The measurements of the vertical and anteroposterior thickness of the condylar cartilage were obtained and the means and standard deviations were determined. The significances of the means of the differences in the thickness of the condylar cartilage were determined by employing the Student "t" test.

RESULTS

The thirteen rabbits comprised of eight in group one and five in group two, consumed rabbit chow, fresh vegetables and water freely, during the experimental periods. They appeared healthy and their chewing function also appeared normal.

The gross appearances of the experimental and control condylar articular surfaces were glistening and smooth. (Fig. 2.)

Microscopically, both the experimental and control condyles were normal having a thin layer of collagen and fibrocytes overlying the highly cellular perichondrium (Fig. 3 and Fig. 4) The subperichondrial tissue showed a normal pattern of differentiation to chondrocytes producing intercellular cartilage matrix. The cartilage showed calcification and resorption with surface apposition of osteoid and bone at its junction with spongy bone of the mandible (Fig. 5).

CELL DENSITY MEASUREMENTS AND LABELING INDICES OF THE PERICHONDRIUM (GROUP 1)

The cell density mean of the perichondrium of the exprimental condyles was 229.39 ($\pm$ 10.90) per 0.25 mm^2, significantly greater than the mean of the control value of 219.05 ($\pm$ 8.70) per 0.25 mm^2 for $P < 0.05$. (Table 9).

(Fig. 3).

The mean labelling index of the perichondrium in the experimental condyles was 19.83 (± 4.57) per 0.25 mm^2, greater than the control value of 15.92 (± 4.46) per 0.25 mm^2, but statistically was not significantly different for $P < 0.05$. (Table 10) (Fig. 4)

CELL DENSITY MEASUREMENTS AND LABELLING INDICES OF THE CARTILAGE (GROUP 2)

The cell density mean of the chondrocytes in the cartilage of experimental condyles was 67.45 (± 10.51) per 0.25 mm^2 , not significantly different from the control value of 67.76 (± 8.12) per 0.25 mm^2 for $P < 0.05$. (Table 16).

It was observed in the autoradiograms that the silver grains overlying the nuclei in the perichondrium were more dense and numerous (Fig. 3), whereas those in the chondrocytes of the mature cartilage in group 2 were diluted, by comparison (Fig. 5).

The mean of the labeled chondrocytes in the cartilage of the experimental chondyles was 3.59 (± 1.52) per 0.25 mm^2, greater than the control value of 2.49 (± 1.17) per 0.25 mm^2, but not significantly different for $P < 0.05$. (Table 17)

THE VERTICAL AND ANTEROPOSTEIOR CONDYLAR THICKNESSES:

The measurements taken of the vertical thickness of the condylar cartilage included both the perichondrium and the cartilage. The thickness of the perichondrium in the experimental condyles was 79.48 (± 30.42) microns, greater than the mean control value of 74.76 (± 33.31) microns, but not significantly different at the P=0.05 level. Similarly, the mean vertical thickness of the experimental cartilages was 256.26 (± 70.22) microns, greater than the mean control value of 228.29 (± 33.69) microns, but not significantly different for $P < 0.05$ (Table 31).

The mean A-P measurement of the experimental condyles was 4276.87 (± 1018.84) microns, not significantly different from the control value of 4154.93 (± 762.05 microns (Table 45).

COMPARISON OF MEANS OF TWO SAMPLES OF CELL DENSITY, LABELLED CELLS, VERTICAL CONDYLAR THICKNESS, AND A-P MEASUREMENT IN T199 AND SALINE TREATED CONDYLES OR RABBIT, BY THE "t" TEST

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - 0}{\sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

$$s^2 = \frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{2N - 2}$$

Cell Density of Perichondrium

$s_{exp.} = 10.90$ $s_{con.} = 8.70$

$s^2 = 97.25$ $t = 20.970$

Labelling Index of Perichondrium

$s_{exp.} = 4.57$ $s_{con.} = 4.46$

$s^2 = 20.39$ $t = 1.7320$

The critical t value with 14 degree of freedom for P=0.05 is: t=1.761

Cell Density of Cartilage

$s_{exp.} = 10.51$ $s_{con.} = 8.12$

$s^2 = 87.92$ $t = -0.0522$

Labeling Index of Cartilage

$s_{exp.} = 1.52$ $s_{con.} = 1.17$

$s^2 = 1.87$ $t = 1.2718$

The critical t value with 8 degree of freedom for P = 0.05 is: t=1,860.

Cond. Thickness of Proginitor L.

$s_{exp.} = 30.42$ $s_{con.} = 33.31$

$s^2 = 1017.34$ $t = 0.4013$

Cond. Thickness of Cartilage

$s_{exp.} = 70.22$ $s_{con.} = 45.46$

$s^2 = 3498.73$ $t = 1.197$

The critical t value with 24 degree of freedom for P = 0.05 is: t=1.711

A-P Measurement of Condyle

$s_{exp.} = 1018.84$ $s_{con.} = 762.05$

$s^2 = 809377.57$ $t = 0.346$

The critical t value with 24 degree of freedom for P - 0.05 is: t=1.711

DISCUSSION

The purpose of early experiments in studying the synthetic nutritive media for the maintenance of cells in vitro was to devise an optimal concentration of nutrients to maintain and support cell survival and growth. Currently, the synthetic media are composed of chemically-defined substances containing many necessary factors for the maintenance of cell life in vitro. The use of synthetic tissue culture medium T199, established by Morgan (1950), contains the essential elements for cell growth and survival when supplemented with 10% serum. T199 has been used by many investigators to support growth of animal cells both in vitro (Morgan 1958) and in vivo (Toto 1968, Bayarado 1977, Haupers 1978, and Eastman 1980), and experimental joint explants (Ray, 1972).

The conveyer of genetic information from one generation to another, DNA, is synthesized during the chromosomal replication, S-phase, prior to mitotic division. Thymidine, a DNA precursor, when labeled by the isotope tritium can be incorporated into the cells undergoing DNA synthesis. Therefore, the rapidly proliferating cell system uptake of tritiated thymidine as seen in autoradiograms is accepted as an index of proliferative activity of the tissue of articular joints, adjacent to the subcutaneous administration of T199 and saline solutions.

After mitotic division, the daughter cells enter the postmitotic phase G_1 when about 50% recommit the cell cycle while the others perform the functional activities characteristic to the differentiation of the cell. In this phase the nutritional and other microenvironmental factors are considered to be of great significance in regulating the growth rate of these cells.

Because of the matobolic stability of DNA, the radioactivity is distributed evenly between the daughter cells labelled with tritiated thymidine and the label is carried out through a number of cell generations. Therefore, the label concentration in the nuclei is diluted in each cycle which was clearly seen during the examination of early labelled perichondrium in the condyles of animal specimens taken after 3 hours in group 1, and those in the cartilage taken 7 days following tritiated thymidine injection in group 2 (Fig. 5).

Histologically, three zones or layers can be easily distinguished (Fig. 6); a superficial articular layer formed of spindle fibroblasts and fibrous tissue; and intermediate zone formed of dense oval cells, where the labelled nuclei were more abundantly distributed than the other zones. This zone is considered as the progenitor or growth pool layer, where the cells proliferate and differentiate into chondrocytes or chondroblasts, which proceed to produce cartilage matrix giving rise to the hypertrophic zone. In this zone

the cells and intercellular matrix undergo all the changes advancing to the calcified cartilage and bone replacement of the carilage, which is seen at the junction between the cartilage and bone. These findings are similar to those of Blackwood (1966) and Lauder (1982).

The significant increase in cell density of the perichondrium of the experimental condyles suggests that either the cells of the proliferating population are closely packed or there is an increase in the generative population of cells. This suggestion is supported by the increase in the number of tritiated thymidine labelled cells in the perichondrium of the experimental condyles (Fig. 3). The diference in the labelling indices between the perichondrium of the condyles treated with T199 and saline (Fig. 3 & 4) represents the premitotic activities for each group, after 24 hours of last injection of both solutions. An increase in the labelling index strongly suggests that there is probably a recruitment of unscheduled cells into the S-phase of DNA synthesis. This could be interpreted as an amplification of the growth rate in the perichondrium of an already active condylar growth of the 6 week old rabbits.

The postulated increase in growth rate can be supported by the increase in the labelling index in the cartilage observed in group 2. Such labelling could be provided by the increased labelled cells in the perichondrium seen in group 1. As the perichondrial cells enter the cartilage,

they migrate and differentiate into chondroblasts, such cells do not undergo mitosis, therefore, they may represent the increased labelled population of perichondrial origin.

Further support of this suggestion is provided by the increased vertical thickness of the cartilage including both thicknesses of the perichondrium and the mature cartilage. The principal growth directions of the vertical columns of the chondrocytes provide for such an increase in the vertical thickness. Moreover, as there is no differences in the size of the chondrocytes in both groups as suggested by equivocal cell density, the probable source of growth could be the increase in the cell population, as a result of increased mitotic activities as a function of the tissue culture medium T199 admininstered daily into pariaticular subcutaneous pouches of young growing rabbits for 7 days.

The mechanism of T199 stimulation of the cells in the perichondrium is not known. However, two possible explanations seem appropriate to suggest. The T199 may directly come in contact with plasma membrane of the perichondrium and compete with those molecules of the nutrients provided by normal diffusion through the synovial membrane, or simply supplement such natural sources of nutrients. It may be possible that one or more of the components in T199 upon contact with the plasma membrane induces the cell to enter into unscheduled DNA synthesis. This could explain the increase in the number of cells labelled with tritiated

thymidine. Although, the observed increase in the labelling index is not statistically significant at 0.05 level, it is possible that increasing the frequencey of administration of 1% T199 or increasing its concentration or both might produce a significant labelling index. This is indicated as the near significance suggested by the findings indicated need for additional studies.

However, this interpretation of unmodified 1% T199 is not consistent with the effect of T199 upon growth of cells in vitro which is dependent upon the supplemented 10% calf serum. The serum is known to contain growth factors, in addition to the nutrients. Therfore, it is more likely that when used in vivo, T199 not only supplements the nutrients carried by the blood to the microenvironment of the perichondrium, but like natural nutrients, is dependent upon serum growth factors also supplied by blood (Lumbach 1976, Antonaide 1979, Heldin 1979, and Ross 1971). Provided growth factor already are available to the microenvironment of the perichondrium, the cells may be primed to utilize the supplemented nutrients provided by T199, for the induction of unscheduled DNA synthesis.

The latter explanation is supported by the information that the perichondrium in the mandibular condyles of six week old rabbits is rapidly growing, and that the growth of cartilage is dependent on growth hormone. This consideration suggests that in another experiment utilizing this

model, T199 may be itself modified by the addition of growth factors such as growth hormone, fibroblast growth factor, or epidermal growth factor and again to measure the effect on the frequency of the labelling of the cells with tritiated thymidine.

The mechanism of parenteral nutrition may be similar to that of the systemic intake of growth promoting elements used in the intravenous feeding hyperalimentation, as has been proved by Dudrick et al. (1968). This was supported also by other investigators, Himes (1978); Nakamato (1981); Sara (1976) and Srivastava (1972), studying the effect of malnutrition on growth and development, showing results that strongly suggest malnutrition causes retardation in growth of mandible and long bones. Pucciarelly (1980) stated that under certain experimental conditions a nutritional factor may stimulate in the mouse cranial differentiation to an extent greater than that brought by the genetically controlled factor. However, it is difficult to specify any certain nutrient factor that is responsible for these growth effects.

The observations of the significant increase in cell density and increased labelling index in the experimental perichondrium of the condyles in group 1; the increased labelling index in the experimental cartilage of the condyles in group 2; and the increased condylar thickness of both perichondrium and cartilage of he T199-treated con-

dyles, all suggest an increase in growth amplification in growing rabbits. This view supports the findings of Toto (1968), Bayardo (1977); Haupers (1978), and Eastman (1980) of in vivo growth amplification. In group 2, where the animals were left 7 days alive after the administration of the tritiated thymidine, the labelling concentration in the chondrocyte nuclei was diluted through the following cell cycles during that period. Therefore, serial or daily sacrificing of animals is recommended to obtain more accurate results.

SUMMARY AND CONCLUSION

Thirteen young New Zealand five to six week old rabbits were used. 5 cc of air was subcutaneously injected over the right and left tempromandibular joint capsules in each rabbit. The right and left capsules were randomly assigned for experimental and control groups. After 24 hours, 5 cc of 1% tissue culture medium T199 or saline (0.85%) was injected into the pouches of the experimental and control group respectively. This procedure was repeated daily for 7 days.

Twenty-four hours of the last injection, the animals were injected intra-articularly with 100 uCi of tritiated thymidine for each capsule. Eight animals (group 1) were sacrificed after 3 hours and 5 animals (group 2) 7 days following the administration of thymidine.

Histological sections of all condyles were prepared for autoradiograms. Autoradiographic analysis of the condyles showed a significant increase in cell density and an increase in labelling index in the perichondrium of the experimental condyles in group 1. Also, an increase in the labelling index in the cartilage of the experimental condyles in group 2 was observed as compared to the control condyles. Vertical measurements of the perichondrium and cartilage layers showed a slight increase in T199 treated condyles, while the anteroposterior measurements showed no

change in the experimental condyles as compared to their controls.

The results of this study suggest that the local administration of 1% artificial tissue culture medium T199 has a tendency to stimulate growth of the mandibular condyles in growing rabbits. Also, the findings strongly suggest that the condylar growth might be modified by enhancement with local administration of enriching nutrients.

A new study may be designed to increase the suggested positive effects of T199 noted in this study. This could include an increase in the frequency of administration of T199, varied by increasing its concentration and volume. Furthermore, the effect of growth factors may be measured by administering T199 with and without growth hormone, fibroblast growth factor and epidermal growth factor. As the tritiated thymidine distribution is diluted following mitotic division in the cartilage, a reduction in the time to daily intervals selected for animal sacrifice is indicated. This could provide a better sampling to determine the rate of migration of prechondrocytes to the subperichondrial cartilage of the condyle.

BIBLIOGRAPHY

1. Antoniades, H., Scher, C., Purification of Human-derived growth Factor, Proc. Natl. Acad. Sci. USA 76 (4): 1809-13, 1979.

2. Bayarado, R., Growth Stimulation with Tissue Culture Medium on Suture of Albino Mice, Thesis at Loyola University, 1977.

3. Bell, W.E., Clinical Management of Temporomandibular Disorders. Chicago Year Book Publisher, 1982.

4. Bhaskar, S.N., Growth of the Rat Mandible from 13 Day Insemination Age to 30 Days After Birth. Am J. Anat. 9:1, 1953.

5. Blackwood, H.J.J., Growth of Mandibular Condyle of the Rat Studied with Tritiated Thymidine. Arch. Oral Biol. 11:493, 1966.

6. Buchner, R., Induced Growth of the Mandibular Condyle in the Rat. J. Oral Rehabil. 9:7, 1982.

7. Carlson, D.S., McNamara, J.A. (Jr.), & Taul, D.H., Histological Analysis of the Growth of the Mandibular Condyle in the Rhesus Monkey. Am. J. Anat. 151:103, 1978.

8. Dooner, J. & Porter, K., Use of Thymidine -2- 14C in the Study of Bone Formation in Vitro. J. Dent Res. 55: 1102, 1972.

9. DuBrul, E.L., Sicher's Oral Anatomy, Mosby Co., 7th ed., St. Louis, 1980.

10. Dudrick, S.J., Wilmor, D.W., & Rhoads, J.E., Long-Term Total Parenteral Nutrition With Growth, Development, and Positive Nitrogen Balance. Surger. 64:134, 1968.

11. Eastman, L.B., Alloplastic Implantation in Primates Calscorbate-Culture Medium T199. A Sequential Histological Study. Thesis at Loyola University, 1980.

12. Folk, L.E.A. & Stallard, R.E., Cellular Kinetics within the Mandibular Joint. Acta Odontal. Scand. 25:437, 1967.

13. Garcia, E., Stimulation of Growth in the Suture of Albino Mice. Thesis at Loyola University. 1979.

14. Glimcher, M.J., Experimental Studies of Transplantation of Joints. In: The Hip Proc. of the First Open Scientific Meeting of the Hip Society. St. Louis, Mosby Co., 1973.

15. Haupers, P., Culture Medium T199 Alloplastic Implant in Primate. A Sequential Histological Study. Thesis at Loyola University, 1978.

16. Heldin, C.H., Westermark, B. & Wasteson, A., Platelet-derived Growth Factor. Proc. Natl. Acad. Sci USA. 76 (8); 1809-13, 1979.

17. Himes, J.H., Bone Growth and Development in Proteins-Calorie Malnutrition. Wld. Rev. Nutr. Diet. 28:143, 1978.

18. Kanouse, M.D., Tanifjord, S.P. & Nasjleti, C.E., Condylar Growth in Rhesus Monkey. J. Dent. Res. 48:1171, 1964.

19. Kiely, M.L., Domm, L.V., The Effect of a Magnesium Deficient Diet and Cortisone on Growth of the Rat Incisor. J. Dent. Res. 56:1577, 1977.

20. Kornbert, A., The Synthesis of DNA. Sci. Am. 214:64-18, 1960.

21. Lembach, K., Induction of Human Fibroblasts Proliferation by Epidermal Growth Factor (EGF). Proc. Natl. Acad. Sci USA - 73(1): 183-187, 1976.

22. Lester, K.S., Ash, M.M., (Jr.)., & Lillie, J.H., Condylar Cartilage, a Scanning Electron Microscope Study of Anorganic Mammalian Condyles. J. Prosth. Dent. 45:422, 1981.

23. Luder, H.U., Weilenmann, W. & Schroeder, H.E., Application of Morphometerical Methods in Studies of the Mandibular Condyle. J. Microscope. 123:371, 1981.

24. Joondeph, D.R., An Autoradiographic Study of Temporomandibular Articulation in the Growing Monkey. Am. J. Orthodont. 62:272, 1972.

25. McAnulty, P.A. & Dickerson, J.W.J., The Development of the Weanling Rate During Nutritionally Induced Growth Retardation and During Early Rehabilitation. Br. J. Nutr. 32:201, 1974.

26. Mankin, H.J., Localization of Tritiated Thymidine in Articular Cartilage of Rabbit. Growth in Immature

Cartilage. J. Bone Joint Surg. 44A:682, 1962.

27. Ibid, Bone Joints Surg. 45:529, 1963.

28. Mike, A., Atoji, V., Fujimoto, E., Kikue, Y. & Mizoquti, H., A New Method to Perform Autoradiography Using 3H-Thymidine and Histochemical Demonistration of Acetylcholinesterase Activity on the Same Section, Kaibogaku Zasshe. 56:313, (Eng. Abst.).

29. Morgan. J.F., Morton, H.J., & Parker, R.C., Nutrition of Animal Cells in Tissue Culture. I. Initial Study on Synthetic Medium. Proc. Soc. Exp. Biol. Med. 73:1, 1950.

30. Moss, M.L., The Primacy of Functional Matrices in Orofacial Growth. Practitioner, 19:65, 1968.

31. Moss. M.L., & Rankow, R., The Role of Functional Matrix in Mandibular Growth, Angle Orthodont. 38:95, 1968.

32. Moss, M.L. & Simon, M., Growth of Human Mandibular Angular Process. A Functional Cranial Analysis. Am. J. Phys. Anthrop. 38:127, 1968.

33. Moss, M.L. & Salenlijn, L., The Primary Role of Functional Matrices in Facial Growth. Orthod. 55:566, 1969.

34. Mott, W.J., Toto, P.D., Hilgeres, D.C., Labelling Index and Cellular Density in Palatine Shelves of Cleft Palate Mice. J. Dent. Res. 48:263, 1969.

35. Nakamato, T., Malnutrition and Development of Mandible and Long Bone in the Rat. Nut. Rev. 39:142, 1981, (4 ref.)

36. Oberg, T., Fajers, C.M., Friberg, U. & Lohmander, S., Collagen Formation and Growth in Mandibular Joint of the Guinea Pigs as Revealed by Autoradiography with 3H-Proline. Acta Odontol. Scand. 27:425, 1969.

37. Ibid, Autoradiographic Studies with 3H-Thymidine on Cell Proliferation and Differentiation in the Mandibular Joint of Young Guinea Pigs, Ondont. Rev. 18:327, 1968.

38. Pollock, J.R., A Comparison Between Rodent and Lagomorph. Thesis at Loyola University, 1962.

39. Pucciarelli H.M., The Effects of Race, Sex, and Nutrition on Craniofacial Differentiation in Rats. A Multivariate Analysis. Am. J. Phys. Anthrop 53:359, 1981.

40. Pucciarelli, H.M., Growth and Functional Components of Rat Skull and Its Alteration by Nutritional Effects. A Multivariate Analysis. Am. J. Phys. Anthrop 56:33, 1981.

41. Ranly, D.M., A Synopsis of Craniofacial Growth. 1st Ed., 1980.

42. Ross, R., Kariya, B., Vogel, A. & Raines, E. Cell Proliferation. Adv. Res. 1:183-187, 1979.

43. Roth, L.J., Stumph, W.E., Autoradiography of Difusable Substances, Academic Press, New York, 1969.

44. Sara, V.R., King, T.L., & Lazarus, L., The Influence of Early Nutritional and Environmental Rearing on Brain Growth and Behavior. Experientia, 32:1538, 1976.

45. Sarnat, B.G. & Wexler, M.R., Growth of the Face and Jaw After Restriction of the Septal Cartilage in the Rabbit. Am. J. Anat. 118:755, 1966.

46. Schoenle, E., Zepf, J., Humbel, R.E., & Froesch, E.R., Insuline Like Growth Factor I stimulites Growth in Hypophysectomized Rats. Nature, 296:252, 1982.

47. Silbermann, M. & Frommer, J., Vitality of Chondrocytes in the Mandibular Condyle As Revealed by Collagen Formation. An Autoradiographic Study with 3H-Proline. Am. J. Anat. 135:359, 1972.

48. Slavkin, H.C., Bringas, P. (Jr.), Cummings, E. & Grodin, M., Initiation of Quail and Mouse Mandibular Chondrogenesis and Osteogenesis in a Serumless, Chemically-Defined Medium., Cal. Tissue Int. 34-111, 1982.

49. Sokoloff, L., The Joints and Synovial Fluid., Academic Press, New York, 1980.

50. Srivastava, U., Vu, M.L., Bhargave, S., & Goswami, T., Metabolic of Nucleic Acids and Protein in the Liver, Brain, and Kidney of Female Rats Subjected to Dietary Restriction During the Period of Gestation as well as the Period of Growth, Gestatin, and Lactation. Can. J. Phys, Pharm. 50:832, 1972.

51. Stockli, P.W. & Willert, H.G., Tissue Reaction in TMJ Resulting from Anterior Displacement of the Mandible in Monkey. Am. J. Orthodont. 60:142, 1971.

52. Toto, P.D., Black, E.E., & Sawinski, V.J., In Vivo Growth Stimulation with Tissue Culture Medium. O.S. O.M. O.P. 25:835, 1968.

53. Vargas, A., Different Concentrations of Tissue Culture Medium in the Suture of Albino Mice. Thesis at Loyola University, 1977.

54. Wagner, A.E., McIlwraith, C.W., & Martin, G.S., Effect of Intra-Articular Injection of Orgotein and Saline Solution on Equine Synovia. Am. J. Vet. 43:594, 1982.

55. Young, R.W., Cell Proliferation and Specialization During Endochondral Osteogenesis in Young Rats. J. Cell Biol. 14:357, 1962.

AVERAGE CELL DENSITY (C.D.) AND LABELLING INDEX (L.I.) IN PERICHONDRIUM OF THE CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #1

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
1-1	230	232	19	13
1-2	236	225	20	18
1-3	220	219	19	12
1-4	265	210	20	12
1-5	269	236	18	16
1-6	275	216	18	14
1-7	249	252	15	10
1-8	227	243	17	14
1-9	225	243	20	16
1-10	213	223	17	12
Total	2409	2299	183	137
Mean	240.9	229.9	18.3	13.7

AVERAGE CELL DENSITY (C.D.) AND LABELLING INDEX (L.I.) IN PERICHONDRIUM OF THE CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #2

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199 R.Cond.	L.I. Sal/ L.Cond.
2-1	245	191	14	14
2-2	288	214	21	10
2-3	222	236	12	14
2-4	242	216	20	9
2-5	257	218	16	9
2-6	230	236	12	8
2-7	234	233	13	11
2-8	248	244	15	10
2-9	209	248	9	9
2-10	198	230	16	14
Total	2373	2266	148	108
Mean	237.3	226.6	14.8	10.8

AVERAGE CELL DENSITY (C.D.) AND LABELLING INDEX (L.I.) IN PERICHONDRIUM OF THE CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #3

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ R.Cond.	L.I. T199 L.Cond.	L.I. Sal/ L.Cond.
3-1	206	231	29	25
3-2	235	229	32	23
3-3	277	228	18	27
3-4	278	241	29	15
3-5	237	240	27	30
3-6	222	234	21	22
3-7	228	231	18	20
3-8	238	227	29	16
3-9	246	226	10	18
3-10	245	215	19	25
Total	2412	2302	232	221
Mean	241.2	230.2	23.2	22.1

AVERAGE CELL DENSITY AND LABELLING INDEX IN PERICHONDRIUM OF CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #4

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
7-1	184	203	19	21
7-2	229	185	16	19
7-3	210	198	23	16
7-4	215	195	31	14
7-5	278	210	24	17
7-6	241	213	33	13
7-7	241	209	22	23
7-8	241	215	17	17
7-9	247	220	19	26
7-10	254	204	29	18
7-11	258	230	27	17
7-12	261	233	28	17
Total	2859	2515	288	218
Mean	238.25	209.58	24	18.17

AVERAGE CELL DENSITY AND LABELLING INDEX IN PERICHONDRIUM OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #5

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
8-1	210	212	21	16
8-2	228	190	23	13
8-3	229	243	25	17
8-4	255	205	17	14
8-5	215	198	20	17
8-6	223	203	27	22
8-7	212	200	20	21
8-8	221	232	20	23
8-9	213	197	22	20
8-10	206	252	19	21
8-11	194	211	12	19
8-12	200	198	23	16
Total	2606	2541	249	219
Mean	217.12	211.75	20.75	18.25

AVERAGE CELL DENSITY (C.D.) AND LABELLING INDEX (L.I.) IN PERICHONDRIUM OF CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #6

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
9-1	212	215	17	15
9-2	225	213	16	13
9-3	230	210	15	12
9-4	210	198	27	16
9-5	223	192	18	13
9-6	227	200	30	14
9-7	231	214	23	23
9-8	215	240	19	16
9-9	220	221	17	22
9-10	209	250	27	18
9-11	222	197	25	22
9-12	240	216	19	21
Total	2664	2566	253	205
Mean	222	213.83	21.08	17.08

AVERAGE CELL DENSITY (C.D.) AND LABELLING INDEX (L.I.) IN PERICHONDRIUM OF CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE PER 0.25mm^2

TABLE #7

Animal & Section Number	C.D. T199/ L.Cond.	C.D. Sal./ R.Cond.	L.I. T199/ L.Cond.	L.I. Sal./ R.Cond.
10-1	214	195	11	7
10-2	220	223	7	10
10-3	203	212	13	8
10-4	240	227	12	10
10-5	228	225	15	14
10-6	201	202	17	9
10-7	245	228	15	9
10-8	242	210	10	7
10-9	210	221	9	6
10-10	206	230	10	7
10-11	208	233	11	12
10-12	230	225	12	6
Total	2647	2631	142	105
Mean	220.58	219.25	11.83	8.75

AVERAGE CELL DENSITY AND LABELLING INDEX IN PERICHONDRIUM OF CONDYLES OF RABBITS TREATED WITH TISSUE CULTURE MEDIUM T199 AND SALINE 0.25mm^2

TABLE #8

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
11-1	228	200	31	16
11-2	220	195	20	20
11-3	212	208	23	19
11-4	207	220	27	23
11-5	202	211	26	22
11-6	225	221	30	17
11-7	222	228	23	21
11-8	230	210	28	16
11-9	207	225	23	15
11-10	215	215	25	19
11-11	227	197	22	18
11-12	218	205	18	16
Total	2613	2535	296	222
Mean	217.75	211.25	24.67	18.50

MEANS OF CELL DENSITY IN PERICHONDRIUM OF CONDYLES OF RABBITS TREATED WITH T199 AND SALINE

TABLE #9

Animal #	C.D./T199	C.D./Saline
1.	240.90	229.90
2.	237.30	226.60
3.	241.20	230.20
7.	238.25	209.58
8.	217.12	211.75
9.	222.00	213.83
10.	220.58	219.25
11.	217.75	211.25
Total	1835.10	1752.36
Mean	229.39	219.05
S.D.	10.90	8.70

THE MEANS OF THE LABELLED CELLS IN THE PERICHONDRIUM OF THE CONDYLES OF RABBITS TREATED WITH T199 & SALINE

TABLE #10

Animal #	L.I./T199	C.D./Saline
1.	18.30	13.70
2.	14.80	10.80
3.	23.20	22.10
7.	24.00	18.17
8.	20.75	18.25
9.	21.08	17.08
10.	11.83	8.75
11.	24.67	18.50
Total	158.63	127.35
Mean	19.83	15.92
S.D.	4.57	4.46

CELL DENSITY AND LABELLING INDEX IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF RABBIT CONDYLES TREATED WITH T199 & SALINE/1.25mm^2

TABLE #11

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ R.Cond.	L.I. T199/ L.Cond.	L.I. Sal/ L.Cond.
4-1	279	319	18	8
4-2	243	328	20	18
4-3	280	345	22	8
4-4	284	336	12	7
4-5	276	337	6	8
Total	1372	1665	78	51
Mean/ 0.25mm^2	54.88	66.60	3.12	2.04

CELL DENSITY & LABELLING INDEX IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF RABBITS TREATED WITH T199 & SALINE/1.25mm^2

TABLE #12

Animal & Secton Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
5-1	352	271	12	15
5-2	384	318	12	15
5-3	344	287	17	7
5-4	442	253	14	7
5-5	392	256	16	9
Total	1914	1388	71	53
Mean/ 0.25mm^2	76.56	55.52	2.84	2.12

CELL DENSITY & LABELLING INDEX IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF RABBITS TREATED WITH T199 & SALINE/1.25mm^2

TABLE #13

Animal & Section Number	C.D. T199/ R.Cond.	C.D. Sal/ L.Cond.	L.I. T199/ R.Cond.	L.I. Sal/ L.Cond.
6-1	398	318	14	12
6-2	409	425	11	5
6-3	396	371	10	2
6-4	366	376	18	8
6-5	404	384	10	6
Total	1983	1874	63	33
Mean/ 0.25mm^2	79.32	74.96	2.52	1.32

CELL DENSITY & LABELLING INDEX IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF RABBITS TREATED WITH T199 & SALINE/1.25mm^2

TABLE #14

Animal & Section Number	C.D. T199/ L.Cond.	C.D. Sal/ R.Cond.	L.I. T199/ L.Cond.	L.I. Sal/ R.Cond.
12-1	325	327	27	19
12-2	354	323	34	22
12-3	334	313	30	26
12-4	332	379	30	10
12-5	364	324	42	16
12-6	325	305	32	26
12-7	353	330	34	29
12-8	267	364	38	23
12-9	278	245	25	23
12-10	307	236	23	22
Total	3338	3315	315	221
Mean/ 0.25mm^2	66.76	66.30	6.30	4.42

AVERAGE CELL DENSITY & LABELLING INDEX IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF CONDYLES OF RABBITS TREATED WTIH T199 & SALINE/1.25mm^2

TABLE #15

Animal & Section Number	C.D. T199/ L.Cond.	C.D. Sal/ R.Cond.	L.I. T199/ L.Cond.	L.I. Sal/ R.Cond.
13-1	292	303	14	10
13-2	313	226	14	6
13-3	287	377	20	8
13-4	319	391	21	6
13-5	300	386	20	28
13-6	298	348	9	8
13-7	285	393	13	17
13-8	295	385	15	18
13-9	301	371	19	15
13-10	292	390	7	11
Total	2987	3770	158	127
Mean/ 0.25mm^2	59.74	75.40	3.16	2.54

THE MEANS OF CELL DENSITY IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE

TABLE #16

Animal Number	C.D./ T199	C.D./ Sal.
4.	54.88	66.60
5.	76.56	55.52
6.	79.32	74.96
12.	66.76	66.30
13.	59.74	75.40
Total	337.26	338.78
Mean	67.45	67.76
S.D.	10.51	8.12

MEANS OF THE LABELLED CELLS IN THE SUBPERICHONDRIAL CARTILAGE FORMING CHONDROCYTES OF CONDYLES OF RABBIT TREATED WITH T199 & SALINE/0.25mm^2

TABLE #17

Animal Number	L.I./ T199	L.I./ Sal.
4.	3.12	2.04
5.	2.84	2.12
6.	2.52	1.32
12.	6.30	4.42
13.	3.16	2.54
Total	17.94	12.44
Mean	3.59	2.49
S.D.	1.52	1.17

CONDYLAR THICKNESSES (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #18

Animal & Sect. Number	Prog.L. T199/ R.Cond.	Prog.L. Sal/ L.Cond.	Cart.L. T199/ R.Cond.	Cart.L. Sal/ L.Cond.
1-1	40	34.5	201	253
1-2	40	40	213	241.5
1-3	46	34.5	184	218.5
1-4	34.5	46	195.5	241.5
1-5	46	46	195.5	207
1-6	40	46	218.5	241.5
1-7	46	34.5	184	230
1-8	46	34.5	184	209
1-9	40	46	190	195.5
1-10	40	40	195.5	230
Total	418.5	402	1961	2267.5
Mean	41.85	40.2	196.1	226.75

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS IN MICRONS OF RABBITS WITH T199 & SALINE

TABLE #19

Animal & Sect. Number	Prog.L. T199/ R.Cond.	Prog.L. Sal/ L.Cond.	Cart.L. T199/ R.Cond.	Cart.L. Sal/ L.Cond.
2-1	34.5	34.5	207	184
2-2	40	40	207	184
2-3	46	40	218.5	190
2-4	34.5	34.5	207	195.5
2-5	40	34.5	207	184
2-6	40	40	213	201
2-7	46	34.5	190	184
2-8	34.5	34.5	207	207
2-9	34.5	34.5	230	201
2-10	40	34.5	195.5	224
Total	390	362	2081.5	1955
Mean	39	36.2	208.15	195.5

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF THE PROGINITOR & CARTILAGE LAYERS IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #20

Animal & Section Number	Prog.L. T199/ R.Cond.	Prog.L. Sal/ L.Cond.	Cart.L. T199/ R.Cond.	Cart.L. Sal/ L.Cond.
3-1	46	45	230	218.5
3-2	46	40	230	195.5
3-3	52	46	241.5	190
3-4	46	46	230	230
3-5	46	34.5	264.5	241.5
3-6	46	34.5	230	230
3-7	46	46	241.5	218.5
3-8	46	46	276	213
3-9	46	46	218.5	241.5
3-10	57.5	46	230	230
Total	477.5	431	2392	2208.5
Mean	47.75	43.1	239.2	220.85

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF THE PROGINITOR & CARTILAGE LAYERS IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #21

Animal & Section Number	Prog.L. T199/ R.Cond.	Prog.L. Sal/ L.Cond.	Cart.L. T199/ R.Cond.	Cart.L. Sal/ L.Cond.
4-1	34.5	57.5	218.5	149.5
4-2	46	46	218.5	161
4-3	40	63	222	144
4-4	40	46	201	149.5
4-5	46	40	201	161
4-6	46	46	195.5	161
4-7	46	57.5	241.5	138
4-8	46	57.5	153	138
4-9	40	34.5	241.5	184
4-10	46	46	218	161
Total	430.5	494	2203	1546
Mean	43.05	49.4	220.3	154.6

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF THE PROGINITOR & CARTILAGE LAYERS IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #22

Animal & Section Number	P.L. T199/ R.Cond.	P.L. Sal/ L.Cond.	C.L. T199/ R.Cond.	C.L. Sal/ L.Cond.
5-1	57.5	34.5	241.5	230
5-2	57.5	34.5	230	230
5-3	69	34.5	218.5	230
5-4	69	40	213	230
5-5	75	46	218.5	195.5
5-6	69	34.5	184	230
5-7	69	34.5	230	218.5
5-8	80.5	46	218.5	230
5-9	69	46	213	218.5
5-10	69	52	184	218.5
Total	684.5	402.5	2151	2231
Mean	68.45	40.25	215.1	223.1

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #23

Animal & Section Number	P.L. T199/ R.Cond.	P.L. Sal/ L.Cond.	C.L. T199/ R.Cond.	C.L. Sal/ L.Cond.
6-1	52	57.5	172.5	161
6-2	57.5	69	172.5	172.5
6-3	69	57.5	161	161
6-4	75	52	138	167
6-5	80.5	46	149.5	161
6-6	80.5	52	149.5	155
6-7	80.5	46	161	161
6-8	75	46	172.5	149.5
6-9	69	57.5	184	172.5
6-10	69	46	161	161
Total	708	529.5	1621.5	1621.5
Mean	70.8	52.95	162.15	162.15

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #24

Animal & Section Number	P.L. T199/ R.Cond.	P.L. Sal/ L.Cond.	C.L. T199/ R.Cond.	C.L. Sal/ L.Cond.
7-1	115	140	287	350
7-2	112.5	150	275	350
7-3	102.5	150	269	300
7-4	100	115	300	325
7-5	100	120	297	330
7-6	106	150	287.5	330
7-7	101	120	281	340
7-8	105	120	275	335
7-9	100	110	269	340
7-10	100	115	275	340
7-11	100	125	275	315
7-12	106	130	281	325
Total	1248	1545	3372	3980
Mean	104	128.75	281	331.66

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF PROCINITOR LAYERS (P.L. & C.L.) IN MICRONES OF RABBITS TREATED WITH T199 & SALINE

TABLE #25

Animal & Section Number	P.L. T199/ R.Cond.	P.L. Sal/ L.Cond.	C.L. T199/ R.Cond.	C.L. Sal/ L.Cond.
8-1	90	100	425	350
8-2	100	105	425	300
8-3	105	110	400	275
8-4	100	105	400	305
8-5	110	110	405	270
8-6	110	115	400	250
8-7	100	105	425	250
8-8	110	100	400	250
8-9	100	100	410	230
8-10	80	95	440	225
8-11	100	100	450	250
8-12	125	100	400	250
Total	1230	1245	4980	3205
Mean	102.5	103.75	415	267.08

CONDYLAR THICKNESSES (VERTICAL MEASUREMENTS) OF PROCINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #26

Animal & Section Number	P.L. T199/ R.Cond.	P.L. Sal/ L.Cond.	C.L. T199/ R.Cond.	C.L. Sal/ L.Cond.
9-1	125	125	275	225
9-2	240	130	300	230
9-3	110	120	300	235
9-4	140	110	310	230
9-5	140	115	310	250
9-6	125	125	325	200
9-7	140	120	300	205
9-8	120	135	325	210
9-9	125	110	300	200
9-10	110	125	330	225
9-11	140	115	305	210
9-12	130	115	300	200
Total	1545	1445	3680	2620
Mean	128.75	120.42	306.67	218.33

CONDYLAR THICKNESSES (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #27

Animal & Section Number	P.L. T199/ L.Cond.	P.L. Sal/ R.Cond.	C.L. T199/ L.Cond.	C.L. Sal/ R.Cond.
10-1	115	100	240	240
10-2	110	105	230	240
10-3	105	100	235	240
10-4	105	100	250	235
10-5	110	90	240	245
10-6	115	110	230	230
10-7	100	95	240	225
10-8	90	95	225	225
10-9	100	100	235	235
10-10	105	90	245	225
10-11	90	100	230	240
10-12	90	100	220	240
Total	1235	1185	2820	2820
Mean	102.92	98.75	235	235

CONDYLAR THICKNESSES (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #28

Animal & Section Number	P.L. T199/ R.Cond.	P.L. Sal/ L.Cond.	C.L. T199/ R.Cond.	C.L. Sal/ L.Cond.
11-1	100	95	350	250
11-2	105	100	350	275
11-3	110	100	345	255
11-4	100	95	355	260
11-5	110	90	350	265
11-6	125	105	360	270
11-7	100	105	390	275
11-8	110	100	360	280
11-9	100	95	375	250
11-10	105	105	355	275
11-11	110	90	350	260
11-12	105	95	360	265
Total	1280	1175	4300	3180
Mean	106.67	97.92	358.33	265

CONDYLAR THICKNESS (VERTICAL MEASUREMENT) OF PROGINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #29

Animal & Section Number	P.L. T199/ L.Cond.	P.L. Sal/ R.Cond.	C.L. T199/ L.Cond.	C.L. Sal/ R.Cond.
12-1	100	95	220	250
12-2	100	90	215	240
12-3	95	90	210	225
12-4	105	75	235	250
12-5	100	85	225	205
12-6	105	100	210	230
12-7	110	85	210	215
12-8	100	90	215	240
12-9	110	95	205	220
12-10	115	90	230	250
12-11	110	100	225	240
12-12	105	100	215	210
Total	1255	1095	2615	2775
Mean	104.58	91.25	217.92	231.25

CONDYLAR THICKNESS (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS (P.L. & C.L.) IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #30

Animal & Section Number	P.L. T199/ L.Cond.	P.L. Sal/ R.Cond.	C.L. T199/ L.Cond.	C.L. Sal/ R.Cond.
13-1	80	65	275	250
13-2	75	65	285	245
13-3	75	70	280	245
13-4	85	60	260	240
13-5	70	60	260	245
13-6	65	65	290	230
13-7	65	60	295	245
13-8	60	70	290	230
13-9	75	65	295	245
13-10	80	65	270	240
13-11	75	65	265	235
13-12	70	70	255	230
Total	875	780	3320	2880
Mean	72.92	65	276.67	240

MEANS OF MAXIMAL CONDYLAR THICKNESSES (VERTICAL MEASUREMENTS) OF PROGINITOR & CARTILAGE LAYERS IN MICRONS OF RABBITS TREATED WITH T199 & SALINE

TABLE #31

Animal Number	P.L./ T199	P.L./ Sal	Cart./ T199	Cart./ Sal
1.	41.85	40.2	196.1	226.75
2.	39	36.2	208.15	195.50
3.	47.75	43.1	239.20	220.30
4.	43.05	49.4	220.30	154.60
5.	68.45	40.25	215.1	223.00
6.	70.8	52.95	162.15	162.15
7.	104	128.75	281	331.66
8.	102.5	103.75	415	267.08
9.	128.75	120.42	306.67	218.33
10.	102.92	98.75	235	235
11.	106.67	97.92	358.33	265
12.	104.58	91.25	217.92	231.25
13.	72.92	65	276.67	240
Total	1033.24	967.94	3331.59	2970.62
Mean	79.48	74.46	256.28	228.51
S.D.	30.42	33.31	70.22	45.46

ANTEROPOSTERIOR (A-P) THICKNESSES OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #32

Animal & Section #	T199/R.Cond.	Sal./L.Cond.
1-1	3584	4480
1-2	3648	4352
1-3	3712	4352
1-4	3584	4480
1-5	3584	4480
1-6	3648	4480
1-7	3584	4416
1-8	3584	4480
1-9	3520	4480
1-10	3684	4480
Total	36096	44480
Mean	3609.6	4448

ANTEROPOSTERIOR (A-P) THICKNESSES OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONES

TABLE #33

Animal & Section #	T199/R.Cond.	Sal./L.Cond.
2-1	3456	3456
2-2	3520	3328
2-3	2520	3328
2-4	2456	3456
2-5	2456	3456
2-6	3392	3456
2-7	3520	3456
2-8	2456	3456
2-9	3520	3584
2-10	3520	3520
Total	34816	34624
Mean	3481.6	3462.4

ANTEROPOSTERIOR (A-P) THICKNESSES OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #34

Animal & Section #	T199/R.Cond.	Sal./L.Cond.
3-1	3328	4096
3-2	3328	4096
3-3	3328	4096
3-4	3328	4032
3-5	3328	4096
3-6	3264	4160
3-7	3328	4096
3-8	3328	4224
3-9	3264	4096
3-10	3200	4096
Total	33024	41088
Mean	3302.4	4108.8

ANTEROPOSTERIOR (A-P) THICKNESSES OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #35

Animal & Section Number	T199/ R.Cond.	Sal/ L.Cond.
4-1	4096	3884
4-2	3968	3776
4-3	4224	3904
4-4	4160	3776
4-5	4096	3884
4-6	4160	3884
4-7	4288	3904
4-8	4160	3904
4-9	4224	3942
4-10	4224	3884
Total	41600	38722
Mean	4160	3872.2

TABLE #36

Animal & Section Number	T199/ R.Cond.	Sal/ L.Cond.
5-1	6080	3840
5-2	6144	3904
5-3	6016	3840
5-4	5760	3776
5-5	5824	3840
5-6	5824	3776
5-7	5888	3737
5-8	5760	3815
5-9	5888	3712
5-10	6080	3763
Total	59264	38003
Mean	5926.4	3800.3

ANTEROPOSTERIOR (A-P) THICKNESSES OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #37

Animal & Section Number	T199/ R.Cond.	Sal/ L.Cond.
6-1	4224	4608
6-2	4224	4800
6-3	4224	4762
6-4	4224	4634
6-5	4224	4608
6-6	4352	4608
6-7	4224	4608
6-8	4224	4736
6-9	4135	4672
6-10	4160	4736
Total	42215	46772
Mean	4221.5	4677.2

TABLE #38

Animal & Section Number	T199/ R.Cond.	Sal/ L.Cond.
7-1	5500	4000
7-2	5437.5	3975
7-3	5500	3987.5
7-4	5525	3975
7-5	5350	3937.5
7-6	5525	3900
7-7	5512.5	3962.5
7-8	5525	4000
7-9	5475	3975
7-10	5475	3912.5
7-11	5187.5	3962.5
7-12	5475	3875
Total	65487.5	47462.5
Mean	5457.29	3955.21

ANTEROPOSTERIOR (A-P) MEASUREMENTS OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #39

Animal & Section #	T199/R.Cond.	Sal/L.Cond.
8-1	4250	3762.5
8-2	4225	3775
8-3	4275	3775
8-4	4212.5	3812.5
8-5	4262.5	3825
8-6	4250	3787.5
8-7	4237.5	3825
8-8	4225	3825
8-9	4262.5	3787.5
8-10	4250	3850
8-11	4225	3837.5
8-12	4250	3837.5
Total	50925	45700
Mean	4243.75	3808.33

ANTEROPOSTERIOR (A-P) MEASUREMENTS OF CONDYLES OF RABBITS TREATED WITH T199 SALINE IN MICRONS

TABLE #40

Animal & Section #	T199/R.Cond.	Sal/L.Cond.
9-1	5000	5000
9-2	4875	4675
9-3	4675	4750
9-4	5000	4837.5
9-5	4650	4850
9-6	5000	4625
9-7	4937.5	4937.5
9-8	4812.5	4675
9-9	4937.5	4875
9-10	4625	4687.5
9-11	5000	4687.5
9-12	4750	4625
Total	58262.5	57225
Mean	4855.21	4768.75

ANTEROPOSTERIOR (A-P) MEASUREMENTS OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #41

Animal & Section #	T199/L.Cond.	Sal/R.Cond.
10-1	3187.5	3187.5
10-2	3250	3125
10-3	3250	3225
10-4	3150	3000
10-5	3162.5	3100
10-6	3125	3125
10-7	3150	3250
10-8	3187.5	3312.5
10-9	3187.5	3250
10-10	3250	3250
10-11	3225	3250
10-12	3275	3250
Total	38400	38325
Mean	3200	3193.75

ANTERIOPOSTERIOR MEASUREMENTS (A-P) OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #42

Animal & Section #	T199/R.Cond.	Sal/L.Cond.
11-1	3625	4437.5
11-2	3678.5	4500
11-3	3750	4500
11-4	3725	4500
11-5	3737.5	4375
11-6	3712.5	4375
11-7	3662.5	4450
11-8	3725	4475
11-9	3650	4462.5
11-10	3725	4450
11-11	3725	4425
11-12	3725	4400
Total	44441	53350
Mean	3703.42	4445.83

ANTEROPOSTERIOR MEASUREMENTS (A-P) OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #43

Animal & Section #	T199/L.Cond.	Sal/R.Cond.
12-1	6125	6125
12-2	6187.5	6250
12-3	6100	6000
12-4	6150	6100
12-5	6137.5	6062.5
12-6	6187.5	6000
12-7	6150	6000
12-8	6125	6062.5
12-9	6125	6250
12-10	6225	6187.5
12-11	6125	6125
12-12	6137.5	6000
Total	73775	73162.5
Mean	6147.92	6096.88

ANTEROPOSTERIOR MEASUREMENTS (A-P) OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #44

Animal & Section #	T199/L.Cond.	Sal/R.Cond.
13-1	3350	3425
13-2	3363	3375
13-3	3263	3388
13-4	3350	3400
13-5	3250	3413
13-6	3250	3275
13-7	3250	3250
13-8	3275	3263
13-9	3263	3375
13-10	3275	3500
13-11	3288	3478
13-12	3313	3375
Total	39490	40517
Mean	3290.83	3376.42

ANTEROPOSTERIOR MEASUREMENS (A-P) OF CONDYLES OF RABBITS TREATED WITH T199 & SALINE IN MICRONS

TABLE #45

Animal #	A-P/T199	A-P/Sal
1.	3609.6	4448
2.	3481	3462.4
3.	3302.4	4108.8
4.	4160	3872.2
5.	5926.4	3800.3
6.	4221.5	4677.2
7.	5457.29	3955.21
8.	4243.75	3808.33
9.	4855.21	4768.75
10.	3200	3193.75
11.	3703.42	4445.83
12.	6147.92	6096.88
13.	3290.83	3376.42
Total	55599.32	54014.07
Mean	4276.87	4154.93

COMPOSITION OF THE TISSUE CULTURE MEDIUM

T199

COMPONENTS

Inorganic Salts	mg/L
$CaCl_2$(anhyd)	140.00
$Fe(NO_3)_3.9H_2$)	0.72
KCI	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

Aminoacids	mg/L
DL-Alpha Alanine	50.000
L-Arginine	70.000
DL-Aspartic Acid	60.000
L-Cysteine HCL-H_2O	0.110
L-Cysteine 2HCI	26.000
L-Glutamic acid	150.000
L-Glutamine	100.000
Glycine	50.000
I-Histidine	21.000
L-Hidroxyproline	10.000
DL-Isoluecine	40.000
DL-Leucine	120.000
L-Lycine HCI	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
DL-Valine	50.000

Other Components	
Adenin sulfate	10.00
Alpha Tocopherol	0.01
Adenylic Acid	0.20
Chloesterol	0.20
Adenosinetriphophate	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

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 HCI	0.025
Riboflavin	0.010

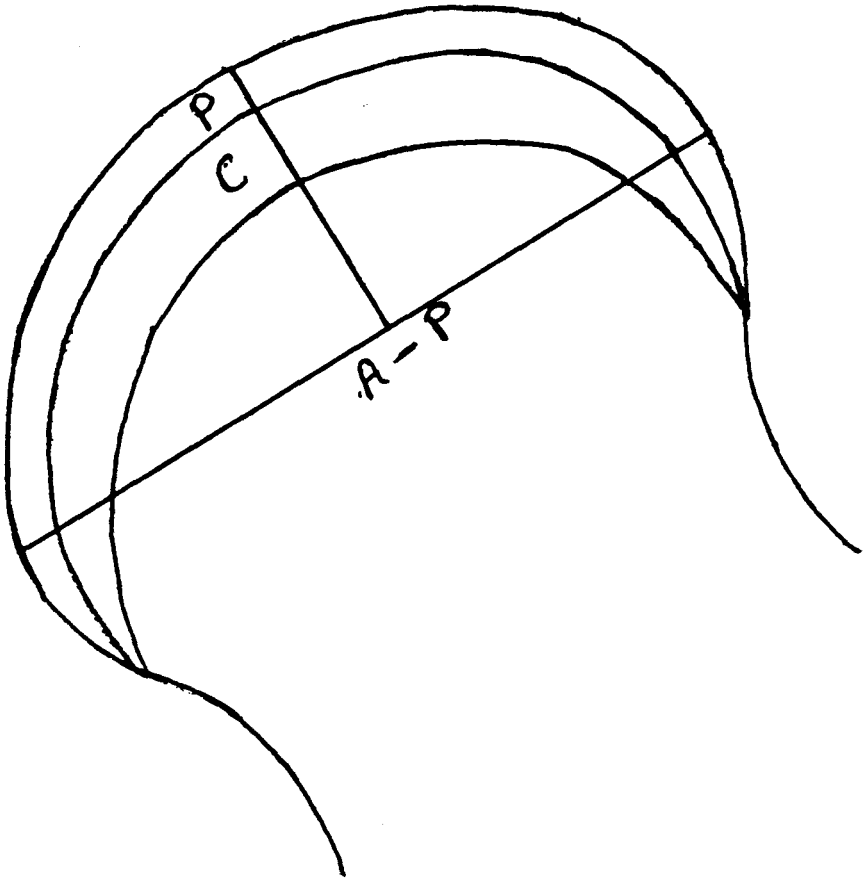

Fig. 1. Diagramatic outline of the mandibular condyle of the rabbit showing the linear measurements including the vertical thickness of the perichondrium (P), the vertical thickness of the cartilage (C) and the maximum antero-posterior measurements (A-P).

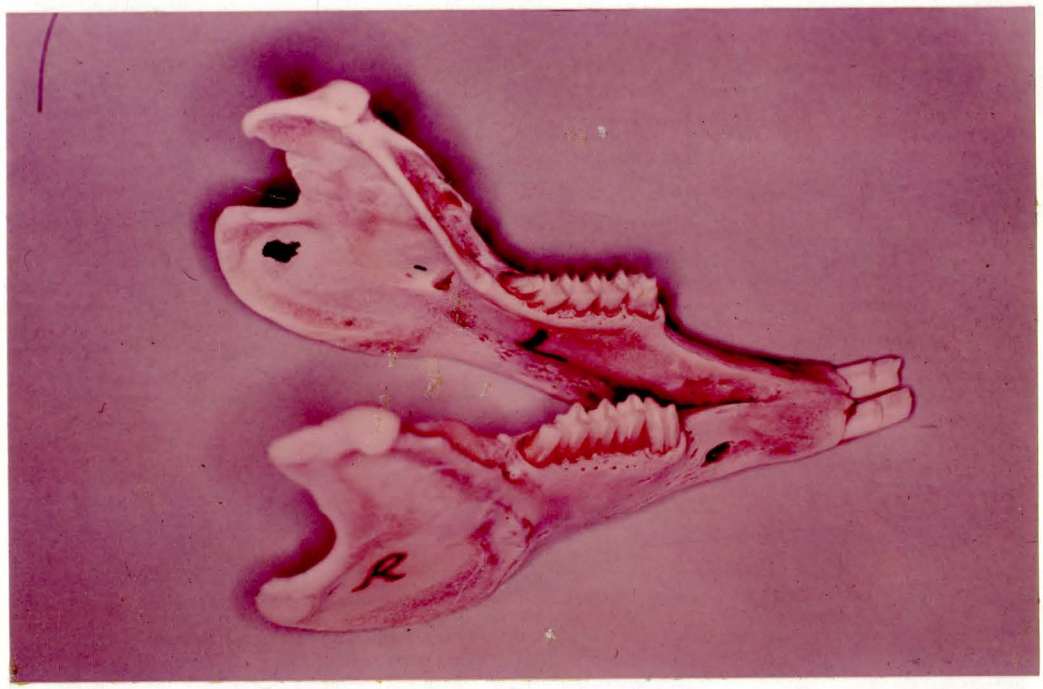

Fig. 2. Whole mandible of an eight week old rabbit showing the anatomical landmarks including the normal mandibular condyles.

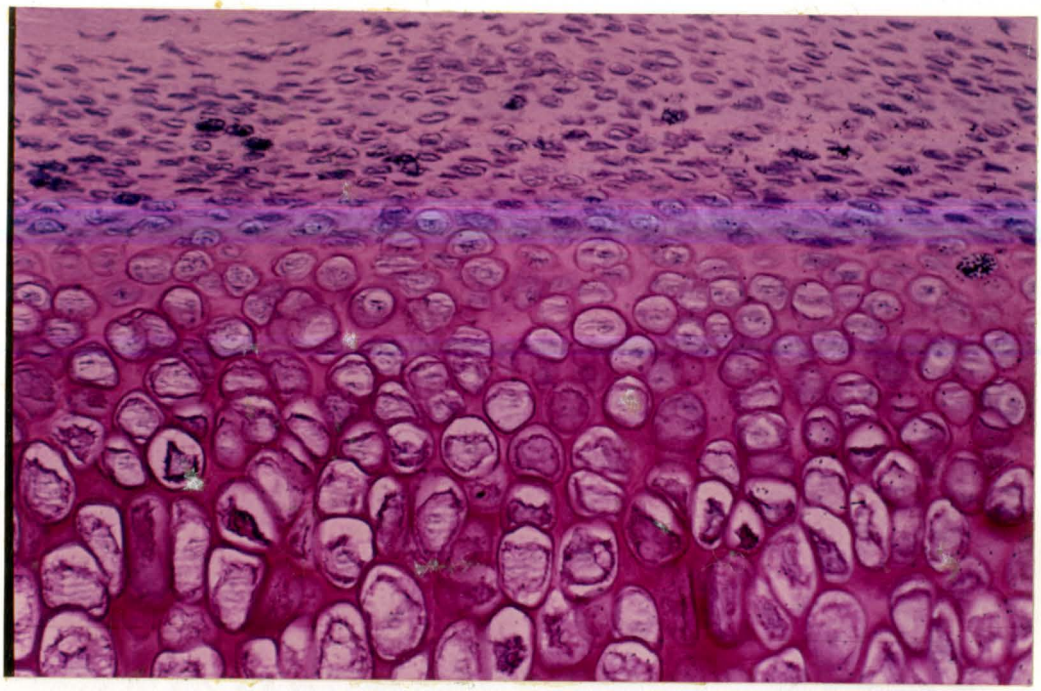

Fig. 3. Section through the mandibular condyle of the rabbit showing tritiated thymidine labeled nuclei in the perichondrium and cartilage of the experimental condyles in group 1. (X 400 original magnification).

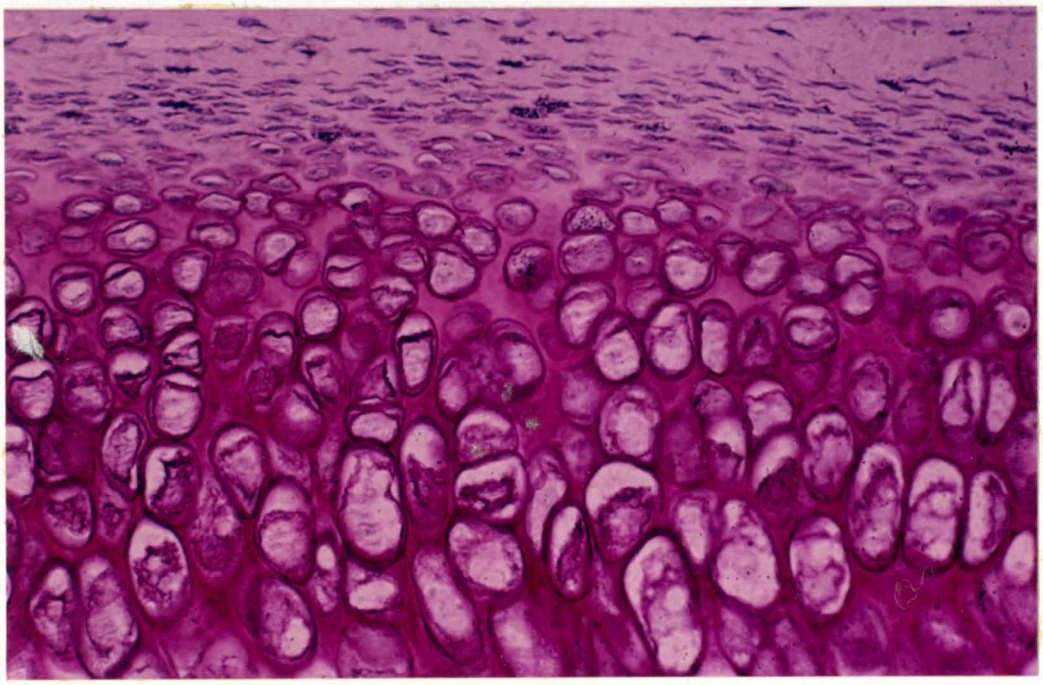

Fig. 4. Section through the mandibular condyle of the rabbit showing tritiated thymidine labeled nuclei in the perichondrim and cartilage of the control condyler in group 1. (X 400 original magnification).

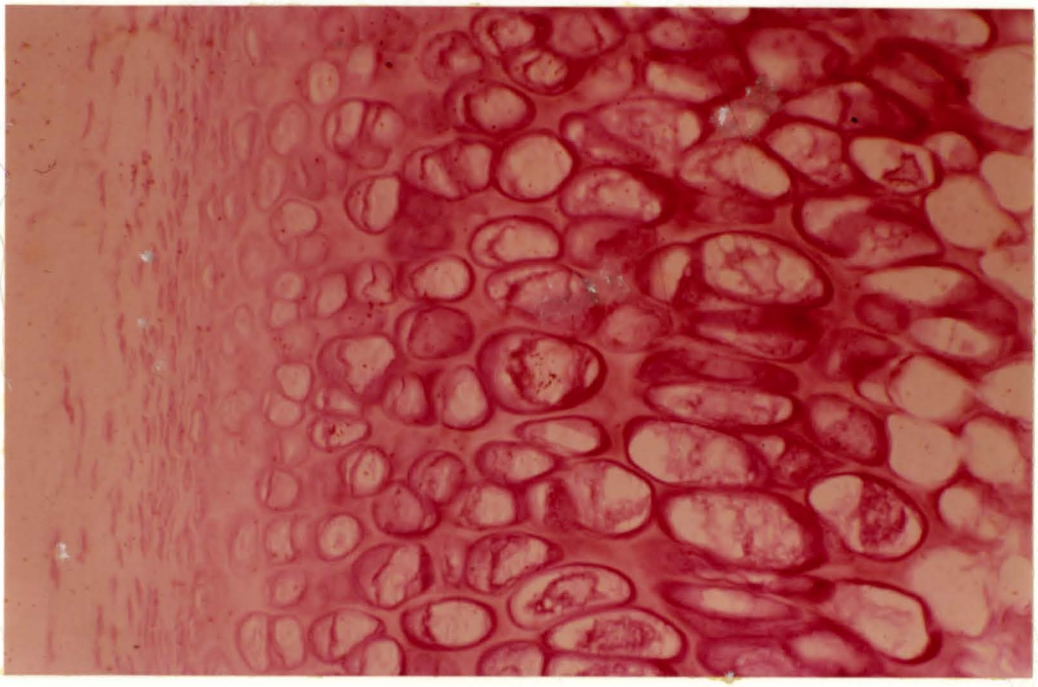

Fig. 5. Section through the mandibular condyle of the rabbit showing diluted tritiated thymidine labelled cells in the cartilage of the experimental condyle in group 2 (X 450 original magnification).

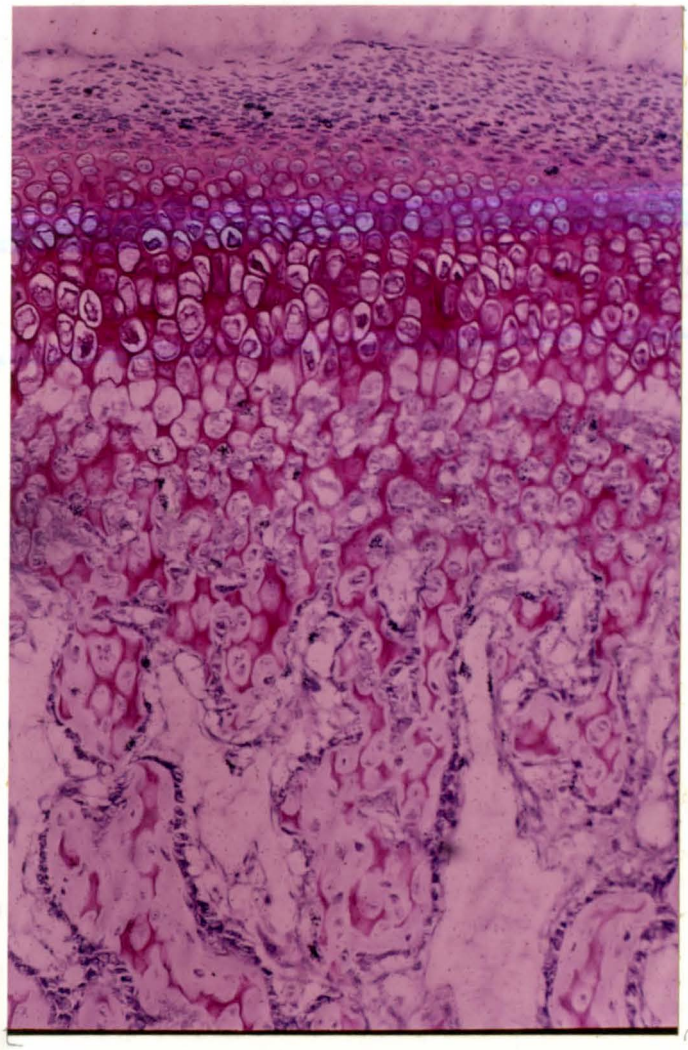

Fig. 6. Section through the mandibular condyle of the rabbit showing the histological layers including: the articular firbous covering (a), the proliferative zone (b) and the differentiating columns of chondrocytes (c) at the junction with bone formation in the condyle (d). (X 100 original magnification).

APPROVAL SHEET

The thesis submitted by Nouraddin Ali Nusier, has been read and approved by the following committee:

Dr. Patrick Toto, Director, Chairman and Professor, Oral Pathology, Loyola University, School of Dentistry, Chicago, Illinois.

Dr. Michael Kiely, Professor, Anatom y, Loyola University, School of Dentistry, Chicago, Illinois.

Dr. Kirk Hoerman, Professor, Preventive Dentistry, Loyola University, School of Dentistry, Chicago, Illinois.

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

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

August 3 1984
Dated

Patrick D Toto DDS.
Director Signature