EFFECTS OF SURGICAL PERIOSTOMY IN ANIMALS UNDERGOING EXPERIMENTAL MANDIBULAR PROTRUSION

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Abstract

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The present investigation was conducted in order to elucidate the role played by the periosteum in regulating growth of he mandible, its condylar process and the condylar cartilage. A bilateral surgical periostomy around the neck of the condylar process was conducted in growing rats and a hyperpropulsion device was bonded onto the upper incisors in order to achieve an anterior repositioning of the mandible . The two procedures (surgery and appliance) were combined in the first group (group I) in order to determine whether the experimental manipulation of the periosteal envelope surrounding the mandibular condylar process modifies in a significant manner growth in animals undergoing experimental mandibular hyperpropulsion. In the second group (group II) only the surgical procedure was performed. In the next group (III) only the hyperpropulsion device was utilized. A fourth group (Group IV) in which only a bilateral sham surgery was performed, served as control.

In each animal, a quantitative evaluation of the dimensional features of the layers forming the condylar cartilage was performed and cell counts were carried out for each layer, at the end of the first two weeks and five weeks of the experimental period. At the end of the first phase, no significant differences in dimensional features were found among groups and

in the cellularity counts the control groups showed values significantly greater than the rest. At the end of the second phase the hyperpropulsion-only group exhibited dimensional values significantly greater than the rest whereas the surgery-only group presented with the greater figures for the cell counts.

The results of our study indicate that the surgical manipulation of the periosteum induced a significant change in the growth parameters evaluated in animals under physiologic or experimental conditions. Therefore, it is conceivable to support the notion that a correlation exists between the periosteal and the cartilaginous component in mandibular growth. Furthermore, the periosteum could act as an indirect local controller of the growth process in the condylar cartilage. Future research should undertake the task of fully characterizing the integrative role played by the periosteum in the regulation of mandibular growth.

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The conclusion of an endeavor such as this study represents the culmination of a project and, hopefully, the beginning of a new one. I would like to extend my sincere appreciation and gratitude to all those individuals that have made a project of this magnitude possible.

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DEDICATION

TO

My parents,

to whom I owe the best of what I am

TABLE OF CONTENTS

Page

ABSTRACT.....ii

ACKNOWLEDGEMENTS.....iv

DEDICATION.....vi

LIST OF TABLES.....viii

LIST OF FIGURES.....xiii

INTRODUCTION.....1

LITERATURE REVIEW.....3

STATEMENT OF THESIS.....40

MATERIALS AND METHODS.....42

RESULTS.....58

DISCUSSION.....109

SUMMARY.....143

CONCLUSIONS.....147

LITERATURE CITED.....150

LIST OF TABLES

Table — Page

IA-1 One-way Analysis of Variance of the mean measurements obtained for the vertical height of the growth zone during phase one.....60

IA-2 Representation of the mean measurements of the vertical height of the growth zone in the different groups during phase one.....60

IA-3 Non-parametric Kruskal-Wallis one-way Analysis of Variance conducted for the mean values of the vertical height of the growth zone during the first phase of the experiment.....61

IB-1 One-way Analysis of Variance of the mean values obtained for the vertical height of the functional chondroblast zone during phase one.....65

IB-2 Representation of the mean measurements of the vertical height of the functional chondroblast zone in the different groupsduring phase one.....65

IC-1 One-way Analysis of Variance of the mean values obtained for the vertical height of the hypertrophic zone during phase one.....68

IC-2 Representation of the mean measurements of the vertical height of the hypertrophic zone in the different groups during phase one.....68

ID-1 One-way Analysis of Variance of the mean values obtained for the vertical height of the total cartilage during phase one..... 70

ID-2 Representation of the mean measurements of the vertical height of the hypertrophic zone in the different groups during phase one.....70

IIA-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the growth zone during phase one.....75

IIA-2 Representation of the mean values obtained for the cell counts carried out in the growth zone in the different groups during phase one.....75

IIB-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the functional chondroblast zone during phase one.....78

IIB-2 Representation of the mean values obtained for the cell counts carried out in the functional chondroblast zone in the different groups during phase one.....78

IIC-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the hypertrophic zone during phase one.....80

IIC-2 Representation of the mean values obtained for the cell counts carried out in the hypertrophic zone in the different groups during phase one.....80

IIC-3 Non-parametric Kruskal-Wallis one-way Analysis of Variance conducted for the mean values of the cell counts of the hypertrophic zone during the first phase of the experiment.....81

IID-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the total cartilage during phase one.....84

IID-2 Representation of the mean values obtained for the cell counts carried out in the total cartilage in the different groups during phase one.....84

IIIA-1 One-way Analysis of Variance of the mean measurements obtained for the vertical height of the growth zone during phase two.....87

IIIA-2 Representation of the mean measurements of the vertical height of the growth zone in the different groups during phase two.....87

IIIA-3 Non-parametric Kruskal-Wallis one-way Analysis of Variance conducted for the mean values of the vertical height of the growth zone during the second phase of the experiment.....88

IIIB-1 One-way Analysis of Variance of the mean measurements obtained for the vertical height of the functional chondroblast zone during phase two.....91

IIIB-2 Representation of the mean measurements of the vertical height of the functional chondroblast zone in the different groups during phase two.....91

IIIC-1 One-way Analysis of Variance of the mean measurements obtained for the vertical height of the hypertrophic zone during phase two.....93

IIIC-2 Representation of the mean measurements of the vertical height of the hypertrophic zone in the different groups during phase two.....93

IIID-1 One-way Analysis of Variance of the mean measurements obtained for the vertical height of the total cartilage during phase two.....95

IIID-2 Representation of the mean measurements of the vertical height of the total cartilage in the different groups during phase two.....95

IVA-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the growth zone during phase two.....98

IVA-2 Representation of the mean values obtained for the cell counts carried out in the growth zone in the different groups during phase two.....98

IVB-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the functional chondroblast zone during phase two.....99

IVB-2 Representation of the mean values obtained for the cell counts carried out in the functional chondroblast zone in the different groups during phase two.....99

IVC-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in thehypertrophic zone during phase two.....100

IVC-2 Representation of the mean values obtained for the cell counts carried out in the hypertrophic zone in the different groups during phase two.....100

IVD-1 One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the total cartilage during phase two.....101

IVD-2 Representation of the mean values obtained for the cell counts carried out in the total cartilage in the different groups during phase two.....101

VA-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean measurements of the vertical height of the growth zone.....104

VB-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean measurements of the vertical height of the functional zone.....104

VC-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean measurements of the vertical height of the hypertrophic zone.....105

VD-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean measurements of the vertical height of the total cartilage.....105

VIA-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean values of the cell counts in the growth zone.....106

VIB-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean values of the cell counts in the functional zone.....106

VIC-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean values of the cell counts in the hypertrophic zone.....107

VID-1 Two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) conducted for the mean values of the cell counts in the total cartilage.....107

LIST OF FIGURES

Figure Page

IA-1. Bar graph depicting the mean measurements of the vertical height of the growth zone during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....62

IA-2. Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the growth zone during phase one in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....63

IB-1. Bar graph depicting the mean measurements of the vertical height of the functional zone during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....66

IB-2. Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the functional zone during phase one in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....67

IC-1 Bar graph depicting the mean measurements of the vertical height of the hypertrophic zone during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....69

IC-2 Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the hypertrophic zone during phase one in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....71

ID-1 Bar graph depicting the mean measurements of the vertical height of the total cartilage during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....72

ID-2 Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the total cartilage during phase one in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....73

IIA-1. Bar graph depicting the mean values for the cell counts in the growth zone during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....76

IIB-1 Bar graph depicting the mean values for the cell counts in the functional zone during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....79

IIC-1 Bar graph depicting the mean values for the cell counts in the hypertrophic zone during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....82

IID-1 Bar graph depicting the mean values for the cell counts in the total cartilage during phase one and phase two in the different groups. The mean values obtained at the end of the first phase are represented by bars on the left (area in black) while the corresponding values at the end of the second phase are represented by bars on the right (area in white).....85

IIIA-1 Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the growth zone during phase two in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....89

IIIB-1 Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the functional zone during phase two in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....92

IIIC-1 Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the hypertrophic zone during phase two in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....94

IIID-1 Bar graph representing the mean values for the measurements of the vertical height and for the cell counts in the total zone during phase two in the different groups. For each group, the measurements are represented by bars on the left (area in black) while the corresponding values for the cellularity are represented by bars on the right (area in white).....96

LIST OF PHOTOGRAPHIC ILLUSTRATIONS

Photo		Page
I	Layers of the condylar cartilage. From the top to the bottom: 1) Resting articular surface 2) Prechondroblst zone 3) Functional chondroblast zone 4) Hypertrophic chondroblast zone 5) Line of erosion	17 ii
I	Mouth holder in place and hyperpropulsor bonded onto the upper incisors	43 ii
II	First contact of lower incisors with hyperpropulsor in movement of closure	43 ii
IV	Mandibular protrusion induced by the hyperpropulsion. Comparison between photo IV and V provides an indication of the amount of protrusion, i.e. 2 mm	43 ii
V	Surgical approach. Initial cut. Parotid gland reflected posteriorly	46 ii
VI	Microseparator exposing the mandibular condyle and part of the ramus	46 ii
VII	A 16x microphotograph of a section corresponding to the hyperpropulsion-only group. Note the good representation of the different layers and the increase in the dimensional features of the cartilage. Cells are loosely arranged	50 ii

VIII A 16x microphotograph of a specimen corresponding to the surgery-only group. Note the compact arrangement of hyperchromatic prechondroblasts in the growth zone. The functional zone has practically disappeared and the hypertrophic chondroblasts are well arranged.....50 ii

IX A 40x microphotograph of a section corresponding to the hyperpropulsion-only group. Note the good representation of the different layers and the increase in the dimensional features of the cartilage. Cells are loosely arranged.....89 ii

X A 40x microphotograph of a specimen corresponding to the surgery-only group. Note the compact arrangement of hyperchromatic prechondroblasts in the growth zone. The functional zone has practically disappeared and the hypertrophic chondroblasts are well arranged.....89 ii

INTRODUCTION

Growth and development of the craniofacial complex has long been a center of attention of exhaustive research. As an important component of the overall process, mandibular growth has been historically considered under the light of diverse hypotheses and various interpretations of distinct nature.

Over the last decades, a growing interest in the regulatory mechanisms controlling mandibular growth has been present in the specialized literature. More specifically, the role of the condylar process in the general development of the mandible has been a continuous source of debate and has generated a considerable amount of investigation.

The interest in understanding the control mechanisms implicated in mandibular growth is twofold. First, knowing more about such mechanisms opens the hypothetical possibility of influencing mandibular growth, which would have a great practical application. In fact, the clinical application through the use of functional appliances, orthognatic intervention and even conventional orthodontic therapy, would be greatly improved with a more precise knowledge of the biological nature of the growth process. The second reason to pursue this line of research can be expressed in terms of a scientific cognitive motivation. Indeed, the elucidation of the basic regulatory mechanisms involved with mandibular growth and, particularly , with condylar activity, would bring us closer to the global comprehension of the phenomena of growth.

Different theories have been repeatedly reported as to the role of the condylar cartilage in the overall process of mandibular growth. From the classic theory of the cartilaginous control, a new trend has evolved which assigns a responsive and adaptatory capability to the condyle. In this perspective, not only the nature of the condylar activity but

also the regulatory factors governing or integrating condylar cartilage activity in the overall process of growth in the craniofacial complex are subjects for investigation.

An increasing amount of scientific information has been incorporated into the field of bone biology concerning the role of the periosteum as a local regulatory factor in the growth process. Interestingly, contradictory results have been reported among similar types of investigations into long bones and the mandibular condyle. The intrinsic interest of the subject as well as the presumed potential clinical value, prompted us to conduct the present research.

The purpose of this study is to elucidate the capability of altering the normal course of postnatal growth that takes place in the condylar process of the mandible through the use of an hyperpropulsion appliance in growing animals. The role of the periosteum in the mentioned process is documented and evaluated experimentally. At the outset it should be pointed out that this study focusses in the connection between the periosteal and the cartilaginous activity in mandibular growth. The outcome of the surgical manipulation of the periosteum or experimental manipulation through the use of hyperpropulsion appliances is evaluated only in terms of condylar cartilage activity. Growth activity taking place in other locations of the mandibular structure is not evaluated and therefore the present work cannot yield the kinds of information essential to determining the relative contribution of the cartilage to overall mandibular growth.

LITERATURE REVIEW

Most of the studies about the mandibular condylar cartilage provide a limited view of its role and function in the overall growth process of the craniofacial complex, in general, or the mandible in particular. Moreover, the purely descriptive approaches commonly utilized to elucidate the growth process have resulted in the generation of a set of ideas which have, for a long time dominated general conceptions of craniofacial development. Morphometric analyses, one of the predominant approaches, deal with the effects of growth, not with its causes . A lack of appreciation of this fact, along with very restrictive notionsof the growth process, contributed to early investigators' thinking that the effects of growth in the mandibular region were a consequence of the changes observed in the condylar region. Such judgements have been partially responsible for the limited ability of clinicians to translate academic knowledge of mandibular growth into successful clinical use.

A general historic overview is mandatory in order to gain certain insight into the general development of the concept of condylar growth and its contribution to facial development.

In this review we will briefly considersome of the classic concepts about the interpretation of craniofacial complex. Likewise, we will review some current notions about growth mechanisms in general and, more specifically, growth activity in the mandibular region. The role thought to be played by the condylar cartilage, as well as the role assumed to be played by the periosteum in the growth process of the condylar process and the evolution of these concepts will be briefly summarized. Various lines of investigation reported recently and methodologically or concveptually connected with the present work will be reviewed.

CLASSIC CONCEPT OF CONDYLAR GROWTH

In one of the first histological studies of mandibular growth dealing with the condyle, Charles (1925) suggested that the temporomandibular joint has an influence on mandibular growth . A few years later, Thoma (1938) again implicated condylar activity in the overall growth of the mandible.

The 1940's saw the beginning of a period of active investigation on the mandibular condyle. Brodie (1941) established that the direction of condylar growth was posterior and concluded that the forward projection of the whole mandible was a consequence of this condylar vector of growth. From this idea, along with the studies of Massler and Schour (1944) showing that the condylar cartilage was a major growth site, the theory developed that downward and forward thrust of the mandible was a result of the growth in the condylar head.

Moore (1949) stated that proliferation of cells in the growth plate cartilage was a major contributor to the growth of long bones. He established a parallelism between this cell proliferation and the production of connective tissue in the head of the condyle.

The studies of Symons (1951, 1952) continue to reinforce the idea of the condyle as a major growth site; meanwhile, in the same years, Sarnat and Engel (1951, 1953) performed the first study of the effects of surgical condylectomies on mandibular growth.

Jarabak (Jarabak et al., 1953) undertook an autoradiographic study concluding that condylar growth was similar to that observed in the growth plate, an idea which was further supported by Levy and Gorlin (1953) in light of several histological observations.

Some other investigators (e.g.Weinmann and Sicher, 1955) adhere to a conceptual trend that can be summarized in words of Sarnat (1957) that "the most important and active center was the condyle, which was the pacemaker and organizer of mandibular growth and

similar, in this role, to the long bone growth plate". These words embody two fundamental notions in a school of thought which holds that the condyle is the major organizer and the agent responsible for the growth process taking place in the mandibular region. As the major growth site in the mandible, the condyle is analogous to the epiphysis of the long bone . Sarnat further reinforced this interpretation in 1963, when he emphasized once again that not only the condyle was the major pacemaker in mandibular growth, but also the equivalent in the mandible to a long bone growth plate.

In 1960, Sicher modified his earlier position (Weinmann and Sicher, 1955) and questioned the adequacy of the parallelism between the mandibular condyle and the growth plate. This modification is an important point of departure in the consideration of the peculiarities of the condyle as a growth site. In spite of this change in view, Sicher continued to assert that the downward and forward growth of the mandible was a result of the upward and backward growth of the condyle.

Some studies of surgical condylectomy, e.g. Jolly (1961) , seemed to support the idea that a strong similarity existed between the condylar cartilage and the growth plate. Baume (1969, 1970) supported this belief and classified the mandibular condyle as a primary growth center. Throughout most of the time period just described, it was believed that the mandible was comparable to the long bone, and that its condylar cartilage was identical in structure and function to the growth plate of the long bone. In this view, the condylar cartilage serves as a major growth center for the mandible. It has been argued by some investigators (e.g. Baume, 1969, 1970) that this persistent perspective has served as a major obstruction to the development of a more complete concept of the growth mechanism involved in the condyle of the mandible. Briefly, it can be concluded from this overview that two basic ideas predominate thinking about mandibular growth. First, the condyle is the major growth center of the mandible, whose upward and backward vector of growth causes the mandible to shift

downward and forward. Second, the mandibular condyle is analogous to the epiphysis of the long bones.

Even though the primacy attributed to the role of the condylar process during growth appears clearly expressed by the classicists, the organizational aspect of its activity does not seem to be sorted out in their interpretation. The coordination of growth responses taking place at other sites of the mandibular structure as a result of the condylar initiative is not specifically clarified. The role of "growth organizer" is acknnowledged for the condyle in the above quotation of Sarnat, but the proper explanation about the operational procedures being carried out (or conducted) in order to achieve this coordination, is missing. Likewise, there is no explanation of how the fine adjustment of changes detected in other locations of the mandibular anatomy is accomplished and directed towards a state of balance and harmony.

Moreover, aforementioned studies uniformly demonstrate a lack of a general concept of growth regulation within the craniofacial complex. Growth activity within the condylar process seems to be considered the main factor , both from a quantitative and regulatory point of view, in the overall mandibular growth. Likewise, growth activity is presented as an independent variable whose correlation with any other parameter is either disregarded or unexplained. If the assumption is made that mandibular growth is mainly regulated by condylar activity, an interpretation must be provided about the nature of stimuli that can trigger, coordinate or integrate this growth activity within the complex of the face. Even if the condyle is a primary regulator growing in an upward and backward direction while the mandible in being displaced in the opposite sense, questions still persists about what induces or controls condylar activity. If a state of functional balance is to be obtained in the overall process of facial development, the general mechanism of local regulatory growth control must be elucidated. The nature of the regulatory linkage between condylar activity and the growth

process in the rest of the structures within the craniofacial complex remains unsolved, and no explanation for these things is given in classic interpretations of the growth phenomena.

These issues are not properly addressed by the classic theory of facial growth and development, even though adequate credit must be given to the basic and fundamental line of investigation conducted over the years in this field. Moreover, it should be acknowledged that dissenting voices were heard in the previous decades, to which, in a strict sense of fairness and scientific interest, we will give attention in the following sections, as we introduce the present perspectives.

CURRENT TENDENCIES: HISTORIC PRECEDENTS

Even though the hypothesis that holds the notion of the condyle as the pacemaker and organizer of mandibular growth prevailed in the orthodontics as the predominant theory for several years, other schools of thought are represented in the scientific literature. For example, as long ago as 1922, Keith and Champion stated that mandibular growth is adaptive in nature, the upper face being the pacemaker within the craniofacial context. They further attributed the capacity of adaptive response to the growth activity of the mandibular condyle. Although the nature of this study was purely descriptive, as the data was obtained from an osteometric analysis on human skulls, the uniqueness of such a perspective at this very early stage should be noted. Conclusions extracted from purely craniometric studies are merely speculative, as they do not provide the kind of evidence that could currently be accepted to support such a hypothesis. Some credit must be given to this and other precedents, specially considering the prevalence at the time of the pacemaker-condyle theory. Nevertheless, it must be kept in mind that other methodological approaches such as cephalometrics are equally descriptive and deal with the effects of growth and not with its causes or mechanisms.

Morant (1936) and Cleaver (1937) both used statistical computations and each concluded that there was no close relationship between the growth activity of the mandibular body and the one of the condyle. This view opened a double controversy that can be expressed as follows. First, there must be more than one major growth site within the mandibular bone, contradicting the assumption that the condylar growth serves as the pacemaker. Second, even if mandibular growth were purely adaptive, the control mechanisms could affect any one of the growth sites. Different factors could be specific regulators of specific growth sites, acting on them independently.

About a decade later, new methodologies began to merge that made it possible to analyze craniofacial growth under a new perspective. For example, hormonal stimulation studies were used to elucidate the specific response of the condylar cartilage.Using this approach, Becks (1946) stated that the condyle and the tibial growth plate reacted differently to growth hormone and thyroxine in animals previously hypophysectomized. In the same year, Collins (1946) studied the effects of the hypophysectomy and concluded that the mandibular condyle and the growth plate cartilages underwent degenerative changes of a different nature from one another.

Exploring the effects of the ablation of the thyroid, Beck et al. (1948) and Baume and Becks (1950) subsequently showed that the mandibular condyle exhibits an independent rate and pace of growth from the mandibular body, as previously stated by Morant (1936) and Cleaver (1937). Importantly, Baume and Becks (1950) showed that the mandibular condyle was less affected than long bones to a thyroidectomy. Also, the study by Baume and Becks, (1950) inaugurated the series of investigations carried out in order to differentiate the specific behavior of the condylar cartilage upon systemic factors in contrast with the response found in growth plate of long bones. Information obtained from this research allowed the generation of

specific behavioural profiles of condylar cartilage in response to systemic and other factors. This will be further discussed in the works of Petrovic.

In 1954, Scott stated that growth of the condyle was adaptive in nature. He accepted the interpretation that the growth vector was upward and backward, but rejected the associated notion that the mandible was thrust downward and forward. Instead, he postulated that the mandibular condyle maintains an anatomic and functional relationship with the rest of the structures of the temporomandibular joint. In order to obtain this effect, the condyle must grow upward and backward while the mandible is being displaced forward and downward by forces other than the condylar growth itself. Scott provided no explanation of the nature of such forces or for the mechanism of mandibular displacement, leaving the most critical issues of the whole process unsolved.

The underlying concept regarding the adaptive character of the condylar growth was initially proposed by Keith and Champion (1922). Moss (1960) subsequently amplified this notion in the development of his functional matrix theory. According to Moss's interpretation , growth in the condyle maintains the temporomandibular joint in a state of functional unity. Therefore, any force that would tend to displace the mandible away from the glenoid fossa during the course of craniofacial development would require adaptive growth of the mandibular condyle, to preserve anatomic and functional balance with the rest of the structures of the joint. In this view, condylar growth, rather than serving as a pacemaker, would be purely compensatory for other phenomena taking place during the development of the face.

Other studies explored differences between the condylar cartilage and the long bone growth plate. For example, after almost twenty years of work, Baume (1950, 1961, 1962, 1969, 1970) concluded that in spite of the many differences that could be found, both the condyle and the epiphysis of the long bone should classify as primary growth centers.

Similarly, Ronning (1967) demonstrated that the mandibular condyle responds differently than the tibial growth plate to papain injections, and Irving et al., (1963) found further differences in studying the response of both the condyle and tibia during scurvy.

The implant studies carried out by Bjork (1955, 1963) questioned many of the classical concepts in growth and development, including notions about condylar growth. From his meticulous analysis of the behavior of the different structures within the craniofacial complex during growth, Bjork (1963) found that condylar growth was adaptive in nature, and that the condylar contribution to the final length of the mandibular body was not significant. These findings portray a mediated and compensatory type of growth for the condyle and minimize its role in the development of the mandible as a whole.

Koski (1968) conducted a study of the growth potential of transplanted components of the mandibular ramus of the rat and concluded that little importance should be assigned to the condyle as a growth site. They further state that its growth activity was mainly involved with the preservation of the functional relationship within the temporomandibular joint. Later studies by Koski (Koski and Mason, 1974), gave further credit to this concept. Some of the classic condylectomy studies (Gianelly and Morrees, 1965) also supported that condylar growth follows the pattern of the adaptive type.

Differences between condylar cartilage and epiphyseal cartilage of long bones in response to metabolic disturbances, like scurvy (Durkin et al., 1968, 1969) or Vit. A deficiency (Baume, 1970) were also investigated and differences were found between changes observed in both the condylar cartilage and the long bone growth plate. These findings were consistent with the notion that a perfect analogy could not be held (or established) between the two structures. The metabolic studies showed further evidence of the remodeling changes that take place in the condyle under modification of the physiologic environment.

The above paragraphs reveal some of the differences in opinions about the two burning questions focussed upon by investigators during the preceeding decades: Should the condylar cartilage be considered a pace maker in the growing face? Does growth of the condylar cartilage play an adaptive role, its growth activity being compensatory and secondary to the changes taking place in other structures of the craniofacial environment?

The second line of investigation tried to elucidate the possible parallelism between the bevavior of the condylar cartilage and the long bone growth plate. This concept was the subject of study from the hormonal, surgical, chemical and dietary points view, and a positive difference was quite clearly established: The condylar cartilage could no longer be considered the pacemaker in the growth process of the mandible, nor had the epiphyseal plate the primary and only role in the growht of the long bone. However, both can be considered analogous, since they play an important role as cartilaginous growth sites in their respective bone structures.

Chronologically, at this point new methodological approaches seemed to be necessary in order to define the specific characteristics of the condylar cartilage and its *modus operandi* in the process of mandibular growth and craniofacial development. Up to this moment, some of the most relevant aspects of the condylar cartilage, such as its embryological origin and development had still not been explored. Likewise, a comprehensive analysis of its response to either systemic regulators or factors from the local environment had not been fully characterized and only isolated pieces of information were available.

In this panorama of well defined and poorly solved dichotomies, the stage seemed to be prepared for a "functional matrix" theory to incorporate the most credible and consistent discrepancies from the classic hypothesis and arise in this scenario of speculation with singular insight. And so, Moss formulated his notion that condylar growth is merely a

response to the functional requirements of the oral viscera, or maybe a reaction to stimuli generated by the orofacial musculature. Following the trend demonstrated by numerous earlier investigators, Moss adhered to the idea that the primary role of the mandibular condyle is to provide the necessary growth to enable the mandible remaining in anatomical and functional contact with the condylar fossa, while the mandible is being carried downward and forward. He characterizes the forces that tend to displace the mandible in that direction as being generated by the surrounding soft tissues.

The functional matrix theory credits the condyle as being a growth site capable of interstitial cartilage- type growth as well as having the capacity for endochondral ossification. However, this theory clearly states that condylar cartilage and long bone growth plate differ in many aspects. Current tendencies support the notion that there is not interstitial component in the growth modality of the condylar cartilage. Condylar growth is presently known to be apositional and follow the pattern of endochondral ossification.

Moss initially incorporated a developmental basis to explain the uniqueness of the condylar cartilage. Other studies have succeeded in displaying the differential characteristics of condylar cartilage in relationship with the growth plate. For example, based on such work, Durkin et al. (1973) observed that "It is obvious that the cartilaginous activities and their involvement in overall growth changes are to be fully appreciated; knowledge of specific growth sites requires basic information concerning the type of growth activity on a cellular level, especially if this knowledge is to be applicable clinically or therapeutically".

The histological descriptions and subsequent analysis carried out by Enlow (1968, 1980, 1982, 1990) provide another view about the role of the condyle in mandibular growth. According to Enlow (1990) most researchers today do not regard the condyle as an structure of unique characteristics which regulates the development of the mandible, including its many parts. Thus, the notion that the condyle serves as a pacemaker of the mandibular

growth, coordinating the activity of all other parts that would be subordinated to it, is out of vogue. Nevertheless, as Enlow affirms, there seems to be a common agreement that the condyle represents a growth site of major importance in the complex mandibular morphogenesis. The condyle functions as a regional field of growth, with certain adaptive capabilities to its immediate environment, to which it responds to accommodate to its localized growth circumstances.The same growth process takes place in all the other regional fields, the growth of the mandible being considered then as "a product of the different regional forces and regional functional agents of growth control acting on it to produce the topographically complex shape of the mandible as a whole" (Enlow, 1990).

In this line of regional adjustments Enlow (1990) includes the different considerations that, over time, have been proposed in order to explain the *modus operandi* of functional appliances. In fact, the amount of pressure exerted over the condylar surface has been considered a major regional factor to which the condyle would respond secondarily.

So, in spite of the intrinsic genetic programming, the rate and direction of growth would be given by extracondylar agents (Enlow, 1990). A relief of pressure upon the condylar surface would result in an increase of growth rate directed towards the articular surface. Likewise, an increase in the amount of pressure would bring about a decrease in the proliferative activity of the condylar cartilage that, in turn, would result in a decrease of growth activity. The level of mechanical stress present upon the condylar surface remains to be properly characterized, and the evidence of compressive forces being exerted on the articular area under physiologic conditions seems to be controversial, according to some investigations (Hylander, 1979). However, the presence of such forces appears consistent with the notion that endochondral growth is pressure adaptive. Therefore, a pressure-resistant cartilaginous mode of growth would be an adaptive response that accommodates the biomechanical environment of the temporomandibular region. Growth in the condylar

process would be possible in spite of loading factors generated by the masticatory apparatus. There are other structural attributes which facilitate growth activity in this location. For example, the hyaline cartilage of the meniscum and capsule consist of fibrocartilaginous tissue, where the fibrous component is tension adaptive and the cartilaginous component is pressure adaptive (Enlow, 1990). Other developmental changes occur as an adaptation to the mechanical requirements. The capsule begins as a vascular tissue but transforms into a non-vascular connentive tissue which provides the temporomandibular joint with the ability to function as a pressure adapted unit. The part played by the loading factors is only a simplified view of the true complex composite of phenomena, brought about by the change in the physiologic environment that takes place upon placement of a functional appliance. It is important to bear in mind, though, that similar mechanisms are advocated by the functional matrix theory.

Based on the functional matrix notion (Moss, 1960), enlargement of the mandible and secondary growth of the condyle are the result of soft tissue development and subsequent mandibular displacement away from the cranial base. Nonetheless, as insistently repeated by Enlow (1990), the entire ramus plays an important adaptive role in the fine adjustment of the mandible to the other craniofacial structures. All parts of the mandible, e.g. the entire condylar process, the coronoid process, the mandibular corpus and its lingual aspect are of considerable importance. Hence, any single approach attempting the challenging task of understanding the mechanisms of facial development should try to comprehend its complexity in the consideration of its multifactorial nature.

DEVELOPMENTAL STAGES OF THE CONDYLAR CARTILAGE

Embryonic development

The temporomandibular joint distinguishes itself by having some peculiarities regarding its embryonic origin and development. In contrast to many other synovial joints, the TMJ is formed by the merging of two intramembranous blastemas, the temporal and condylar blastema (Baume, 1962). While the chronology of the developing components has been summarized by Moffett (1966), for our purposes only the changes and events directly concerned with the cartilage of the mandibular condyle will be mentioned.

At about six weeks in utero, the mandible developing from the mandibular process of the first branchial arch makes its appearance (Levy, 1964; Baume, 1962). At this point, the mandible is in close relationship to the buccal surfaceof the anterior region of Meckel's cartilage on each side of the face (Baume, 1962). Also, around this time condensation of mesenchymal cells at the dorsal end of the primordium of the mandible which forms the condylar blastema begins.

The condylar process does not arise from intramembranous bone formation as the mandibular body and ramus do, nor does it develop from the so-called primary cartilages such as Meckel's cartilage. Rather, it is laid down around the tenth week as a wedge of newly formed cartilage, which undergoes ossification and eventually unites to the body of the mandible at about the 12th week (Baume, 1962). Somewhere between the 10th and 12th week, morphological changes in the condylar process of the developing condylar blastema are noticeable. These changes are the direct result of the formation of the condylar cartilage.

The cartilaginous center seems to be fundamental in the growth of the condylar process (Durkin et al., 1971) ; the adaptive nature of the condylar cartilage starts to show evidence at this point by allowing the condylar process to meet the anatomic relationship with the rest of the developing structures, such as the future glenoid fossa.

To briefly summarize some of these concepts and interpretations, the mandible is essentially a membranous bone in which the condyle develops to meet the demands of a changing situation. Theoretically, pressure is thought to induce chondrogenesis rather than osteogenesis, generating a cartilaginous wedge that will ultimately develop into the condylar cartilage.

Convergent ossification becomes identifiable from the 12th week both in the marginal aspect of the primitive cartilaginous wedge, where it progresses in a downward direction, as well as in the ramus where it takes place in an upward direction. Ossification in the ramus stops at the lateral and posterior aspects of the condylar process where chondrogenesis is taking place (Durkin et al., 1972; Heeley et al., 1970).

By the 15th week, only the center of the condyles are still composed of cartilage (Levy, 1964; Heeley et al., 1970). This is consistent with the idea repeatedly expressed (Enlow, 1990) that endochondral growth occurs only at the articular surface, where chondrogenic differentiation is required given the amount of pressure and mechanical forces. According to this description, the distribution of endochondral bone being laid down in the medullary core of the condylar process in association with the condylar cartilage (Enlow, 1990) and surrounded by periosteal-endosteal bone cortices, is present in the early stages of embryonic development.

Around the 16th week it is possible to differentiate several layers within the condylar cartilaginous cap: a resting zone of outer, flat, undifferentiated cartilage cell s covering the articular surface ; intermediate zone of proliferative activity; a zone of hypertrophic cells , and a zone of erosion. From the 16th week to the end of the embryonic development, cellular organization does not experience major changes. The limits between layers begin to become indistinct but the prominence of the hypertrophic layer is noticeable.

In the postnatal stage, one of the most prominent features of condylar morphology is the architectural arrangement of the chondroblasts. As it has been pointed out by various authors(Durkin, 1972; Enlow, 1979, 1982, 1990; Petrovic et al., 1974), the chondroblasts of a primary cartilage, such as the epyphiseal plate, are usually oriented in parallel rows of cells, while the distribution of cells of a secondary cartilage, such as the condylar cartilage, is much more random in appearance. Besides, as it has been mentioned, the division between the different zones of cells is not as distinct as that seen in the growth plates of long bones. At this point, following on the work of Durkin (Durkin et al., 1973), Petrovic (1982) and Enlow (1990), the stratification of the mandibular condyle can be described as follows:

1-The resting or surface articular zone

2-The transitional of proliferative zone

3-The functional chondroblast zone

4-The hypertrophic zone

5-The zone of erosion

6-The zone of subchondral bone formation

At the most superior region, the cells are arranged with their long axes parallel to the articular surface. These cells define a layer of poorly vascularized tissue covering the articular surface of the condyle, called the capsule. It has been suggested (Enlow, 1990) that this layer represents the chondrogenic derivation of a primitive periosteal coverage forced to undergo histological differentiation under mechanical requirements. Therefore, as previously mentioned, the capsule is originally a vascular periosteal envelope, that transforms into a non-vascular connective tissue under the loading requirements of the articular surface. As proposed by Enlow (1982) , variables levels of compression occur at the articular surface of the mandibular condyle. Since the periosteal mode of growth is not pressure adapted, an

PHOTO I

Layers of the condylar cartilage. From the top to the bottom:

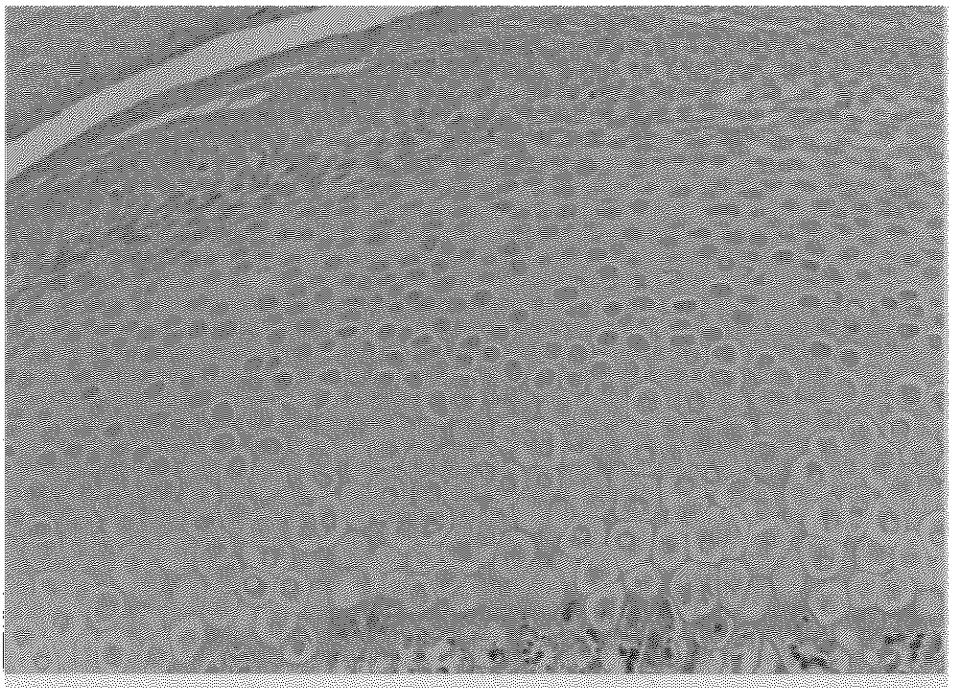
1) Resting articular surface

2) Prechondroblst zone

3) Functional chondroblast zone

4) Hypertrophic chondroblast zone

5) Line of erosion



intramembranous type of growth could not be possible. Therefore, a more pressure resistant endochondral growth mechanism develops at the articular surface of the condyle where pressure exceeds the tolerance of the soft tissue membrane to operate as a growth site. However, it must be taken into consideration from the developmental point of view that only the core of the condylar process is endochondral while the cortex is intramembranous.

It has been discussed for some time whether this capsular layer is equivalent to the resting articular surface of the growth plate (Blackwood, 1966). Current thinking is that the condylar capsule represents a different version , with unique characteristics, from the articular coverage that can be found in the long bone (see for example, Durkin, 1979; Enlow, 1990). The capsular layer is not the source of cellular proliferation, and the cellular activity that takes place in the underlying stratus, is relatively independent of the capsule itself.

The second or proliferative layer, which has been object of intensive investigation over the last decades, consists predominantly of closely packed prechondroblasts cells; the intercellular matrix is practically absent. According to Stutzman and Petrovic (1982) , this lack of a surrounding matrix allows prechondroblasts to be responsive to local factors that, therefore, would be able to modulate the growth rate of secondary type cartilage. In a sense, the prechondroblast would be similar to the preosteoblast.Further, when the prechondroblasts start to produce matrix in the secondary type cartilage, they stop dividing. In primary cartilages , the chondroblasts divide and synthesize intercellular matrix. In this perspective, the presence of intercellular matrix will isolate the dividing cells from any local factors that might restrain or stimulate the cartilage rate growth.

These investigators (Stutzman and Petrovic, 1982) have suggested that the mitotic compartment of secondary cartilage has two cell varieties: the skeletoblast and the prechondroblast. According to Petrovic, the skeletoblast is a fibroblast-like pluripotential stem cell, originating from the embryonic mesenchymal cell. The skeletoblasts are limited in number

but have a potential for a large number of divisions. While they usually differentiate into a preosteoblast, under specific conditions skeletoblasts may differentiate into a secondary type prechondroblasts.

The round-shaped prechondroblasts, derived from the skeletoblasts, are present in larger number, formed into a compact arrangement, and have a reduced mitotic potential (Petrovic, 1982). Prechondroblast multiplication is believed to be regulated by general and local extrinsic and intrinsic factors and the prechondroblast is a differentiated cell that only matures into a secondary- type chondroblast.

Deep to the proliferative layer, a zone of immature hyaline cartilage occurs (Durkin, 1972; Petrovic, 1975, Enlow, 1989). Cells within this zone are larger and more rounded and can be defined as being in a prehypertrophic stage. In this layer no mitotic activity can be found. The cells do not have columnar arrangement and, interestingly, a scant amount of intercellular matrix is noticeable. Without a neat limit, the deeper cells of this layer incorporate themselves into the next layer which is composed of densely arranged chondroblasts, undergoing a process of cellular hypertrophy and surrounded by scant matrix.

In the deepest reaches of the hypertrophied area , the chondroblasts are arranged in a haphazard fashion and separated by a scant amount of matrix. At this level, mineralization occurs in a pattern that circumscribes the chondroblasts. The typical pattern of pericellular calcification is prominent in the lower third of the cartilage but also present in higher levels in the extreme surfaces at an early stage. The hypertrophied cells appear enclosed in a circular wall of mineralized matrix, in contrast with the pattern of calcification in the growth plate cartilage, where mineralization takes place in the vertical cartilaginous partitions between the linear columns of daughter cells (Durkin, 1972; Ronning, 1967). Upon calcification, the mineralized matrix undergoes a process of resorption that is believed to be mediated by

chondroclastic activity, as well as by capillary invasion. As a result, subsequent replacement by endochondral bone takes place.

The pattern of calcification within the cartilaginous component of the mandibular condyle still remains controversial and for some investigators (Durkin et al., 1969, 1973; Silbermann and Frommer, 1972) it does not completely follow the sequence that is accepted for the endochondral type of growth. These authors maintain that the sequence of events is closer to the intramembranous type even at this site, the vascular invasion being secondary to the previous chondroclastic resorption. In any event, subchondral bone formation takes place during the whole growth period of the mandibular condyle, the process involving the resorption of the calcified matrix at the erosion line, allowing the conjunctivovascular buds to penetrate and arrange themselves to formate the endochondral bone trabeculae (Enlow, 1982, 1985, 1989; Thorogood, 1983).

An interactive perspective of the intrinsic regulation of the condylar growth in the proliferative, prehypertrophic and hypertrophic layers was proposed by Petrovic (Petrovic and Stutzman, 1972; Petrovic et al., 1975) who suggests that there is a negative feedback signal originating from the proximal part of the chondroblastic zone and exerting a restraining effect on the prechondroblastic multiplication rate. Any factor inducing either an earlier maturation of the chondroblastic population, as found with thyroxine hormone stimulation will increase the growth rate by stimulating the proliferative process. Likewise, earlier commencement of chondroblastic hypertrophy would lead to decrease of the restraining signal. In other words, as increasing number of cells in the chondroblastic zone undergo hypertrophy, the feedback signal which inhibits the multiplication rate in the prechondroblastic proliferative layer becomes weaker. As a result of this decrease in the restraining signal, an increase in the mitotic activity in the prechondroblastic zone occurs (Petrovic and Stutzman, 1975; 1977) . This complex set of interactions is the mechanism

proposed by Petrovic to explain the "modus operandi" of the mandibular postural hyperpropulsor, as it will be discussed later.

EXPERIMENTS WITH FUNCTIONAL HYPERPROPULSORS-EFFECT OF POSTURAL HYPERPROPULSION ON CONDYLAR GROWTH

The influence of natural forces and functional stimulation on form were first reported by Roux in 1883, when he described the characteristics of functional stimuli involved in building, remodeling and preserving tissue. His functional stimuli hypothesis became the background of both general orthopedic and functional dental procedures.

Haupl in 1945 applied the Roux's hypothesis to the correction of jaw and dental deformities utilizing functional stimuli. Other clinical aspects were almost simultaneously put into use by Andresen (1945).

Since 1945, many other appliances based on the functional stimuli principle have been developed and today, functional appliances have a place of growing importance in the therapeutic armamentarium of the orthodontic clinic. Although clinical effects derived from the theapeutic use of this type of appliance have been reported in the orthodontic literature, the scientific basis that may be used to explain the appearance of these effects still remain partially unknown. Accordingly, it would be of scientific interest to be able to substantiate and better identify, on a biological basis, the intimate mechanisms that govern the effects clinically observed with this type of therapeutic approach.

Although the use of functional appliances seem to be justified because they are clinically advantageous when properly used, an understanding of their *modus operandi* would provide valuable new information that, undoubtly, would increase their range of effectiveness. Furthermore, since the biological mechanisms involved are directly connected with the growth process, their elucidation would improve our understanding of facial

development. With this elucidation in mind, a series of experiments have been conducted over the years by different authors. Of special interest are those which have focused on the effect of mandibular functional hyperpropulsion on the growth of the condyle and the mechanisms involved in the biological response of the condylar cartilage (e.g. Charlier, 1969; Charlier et al., 1969; Elgoyhen, 1972 Petrovic and Stutzman, 1972; McNamara, 1972, 1973; Graber, 1975; Petrovic et al., 1975, 1977, 1981; Stutzman and Petrovic, 1982, 1990) . In these studies the effects of a functional anterior protrusion of the mandible produced in experimental animals were reported. In the series of investigations carried out by Petrovic and his colleages, the design of the hyperpropulsor appliance was such that when the mouth was closed, the lower incisors were propelled forward, relocating the whole mandible in an anterior position. According to Petrovic (Petrovic et al., 1981) "the situation is like if the upper arch were in a more forward position than is actually the case".

The confrontation between the dental arches gives rise to a deviation signal, which is reduced by an appropriate forward positioning of the lower dental arch through the propulsion of the mandible. In this sense, the appliance is passive since it does not provide force by itself, but induces a forward repositioning of the mandible by an accommodation of the muscle function and stretching of the soft tissues.The mechanisms intially suggested by Petrovic and Stutzman pointed towards the preeminence of the lateral pterigoyd muscle as the indirect control factor in the changes observed in growing rats undergoing treatment of hyperpropulsion. Petrovic formulates a hypothesis according to which a prolonged increase in the contractile pterygoid muscle produces an acceleration of growth of the condylar cartilage; a decrease of the muscle activity will slow down the condylar growth. The tension generated by the contractile activity of the lateral pterigoyd muscle was, then, considered one of the factors basically responsible for the growth activity in the mandibular condyle. Tension exerted by this muscle would be either directly responsible for growth stimulation or indirectly

involved in transmitting the growth controlling messages. The appearance of a large series of publications showing the relevance of the role developed by factors of diverse nature (e.g. biomechanical, metabolic, hormonal, physical, biochemical, piezoelectrical) in growth control has forced a revision in thinking about the role of the lateral pterigoyd muscle and necessitated clarification of its hypothetical protagonism in mandibular growth.

Subsequent investigations probing in the nature of the biomechanical stimulation developed by the lateral petrigoyd muscle and its importance in growth of the mandibular condyle provided seemingly contradictory information . An apparent contradiction seems to exist in Petrovic's argumentation, given the fact that upon placement of the hyperpropulsor appliance the mandible is relocated in a forward position and, therefore, the lateral pterygoid muscle is being physiologically shortened rather than stretched. Under Petrovic's perspective, this shortenning of the muscle would carry out a decrease in the growth rate condyle. In fact, the lateral pterigoyd muscle is known to participate in the movements of mandibular closure through its superior head, whereas the inferior portion is active in relation with movements of opening, anterior displacement and laterodeviation of the mandible. When we reposition the mandible forward by placement of a hyperpropulsion device, the inferior head of the lateal pterigoyd is shortened and its physiologic length decreases rather than the muscular structure being stretched. Consequently, the degree of tension present in the muscle decreases as a result of the aproximation of the bony structures where the muscle has its attachments (pterigoyd process and condylar process, for the inferior head). According to the theory supported by Petrovic, a prolonged lack of tension would produce a diminution of the stimulus of growth transmitted to the mandibular condyle.

Experimental findings reported in a subsequent series of investigations (Petrovic and Stutzman, 1972; Oudet and Petrovic, 1978, Petrovic , 1982) related to the histomorphological changes of the lateral petrygoid muscle under mandibular

hyperpropulsion, provide a possible explanation for the apparent contradiction just described: the shortenning of the muscle and secondary decrease of its contractile tension is purely a transitory effect . With prolonged appliance use, the number of serially arranged sarcomeres in the lateral pterygoid muscle decreases. In turn, this reduction induces a change in the anatomical length of the muscle fiber, which is thought to counteract the loss in the physiologic or working length of the muscle secondary to the experimentally induced mandibular protrusion . Thus, the muscle fiber becomes shorter and a return to the conditions of a normal contractile activity can be accomplished (Oudet and Petrovic, 1978). Additionaly , the remaining sarcomeres undergo certain degree of hypertrophy, which allows an increment in the contractile force of the muscle and concomitantly increments in tension transmitted to the condylar process.

A study by McNamara (1973), showed that upon placement of a protrusive appliance in monkeys, a modification of the EMG activity of several muscles of the masticatory apparatus can be detected. As to the lateral pterigoyd muscle specifically, the inferior head did not show any changes, whereas tonic discharges were observed in the superior head, both during postural maintainance of the protrusive position as well as during functional movements. The discharges exhibited a variable frequency that was time related, with the number of tonic impulses peaking between the fourth and the eigth week, when condylar growth experiences its maximum increase. McNamara was unable to discern any causal correlation between the two phenomena (maximum of tonic activity and increased growth of the condylar cartilage), but a temporal correlation was noted. Similar experiments conducted by Petrovic (1981) provided similar results in regard to the temporal correlation between muscle activity and condylar growth. Once again, the causal relationship between these two experimental findings (or phenomena) has not been established. Besides, the histological changes as to the rearrangement of the number and disposition of the sarcomeres present in the muscle are

very slow and do not keep correlation in time with the increment in the growth rate that can be detected shortly after the protrusive appliance is located. On the other hand, it can be speculated that even if the EMG activity generated by the muscle shows an increase in tonic impulses, such activity does not necessarily indicate that increment in the tension is being transmitted by the muscle, beyond the physiologic limits present previously to the placement of the appliance .

Further investigation was carried out by Stutzman et al. (1976) to clarify the relative importance of the superior versus the inferior heads of the lateral pterigoyd muscle and their mode of interaction with another menisco-capsular attachments in the control of growth in the condylar cartilage. The isolated resection of the superior head of the lateral pterigoyd muscle did not result in a significant decrease in the number of mitosis in the condylar cartilage, when compared to control animals. The complete bilateral resection of both heads of the lateral pterigoyd muscle resulted in a significant decrease of the number of mitosis in the proliferative layer of the condylar cartilage, when compared to the figures obtained in controls or in animals where the isolated resection of the superior head was performed.

Even of greater relevance is the finding that the section of the menisco-temporal frenum when both lateral pterigoyd muscles remain intact and functioning resulted in a dramatic decrease of the mitotic activity in the growth zone of the condylar cartilage. The surgical resection of both lateral pterigoyd muscles does not produce any significant effect in growth rate if the menisco-temporal frenum and the rest of of temporomandibular and menisco-capsular soft-tissue structures are experimentally stimulated with a mechanism of passive motion consisting in a mechanical device coupled with the lower joint. According to Petrovic and Stutzman (1981), the retrodiscal pad and capsular apparatus (menisco-capsular attachments) seem to play an important role in condylar growth and represents the link between the lateral pterygoid muscle and its role in the control of condylar growth. In our

perspective, it seems reasonable to propose that the anatomic and functional integrity of the inferior head of the lateral pterygoid muscle is on of several conditions which serve to preserve and maintain the growth process of the condylar cartilage within the limits of physiologic normality .

The effect of the lateral peterygoid muscle (or its inferior head, according to the investigations cited earlier) seem to depend on or be mediated by the action of the menisco-temporal frenum and soft-tissue attachments of the menisco-capsular unit. It has been suggested (Stutzman, 1976; Petrovic and Stutzman, 1978, 1981; De Groote, 1984) that when a forward repositioning of the mandible is carried out by using a protrusive type of device, the inferior head of the lateral pterygoid muscle plays a role of indirect controller of condylar growth activity, acting on the condylar process through the menisco-capsular attachments. The experimental protrusive position would carry out an increment of the tensional level of the capsular structures (or articular structures) with temporo-meniscal or menisco-condylar attachments or insertions.

When the mandible is not immobilized in the experimentally induced protrusive position and functional movements of opening and closure are possible, the range of physiologic mobility results in an additional increment of the tensional gradient transmitted to the condylar process, through the periosteal attachments. Alternatively a redistribution of the elastic forces within the capsular unit could occur in order to reestablish normal levels of elastic traction (or regain mechanical balance) within the temporomandibular region.

It is, therefore, conceivable that the next operative element in the mechanism of regulation of growth in the cartilage of the mandibular condyle is based on the tensional levels developed by the menisco-capsular unit of the temporomandibular joint. The direct effect of the lateral pterigoyd muscle appears minimized in light of the most recent interpretations of the so-called servo-system theory propugnated by Petrovic, although its

contribution is still considered crucial as it has been stated in recent reports (Stutzman and Petrovic, 1990).

The conceptual evolution undergone by the hypothesis of Petrovic et al. presents the lateral pterygoid muscle as an indirect controller that exerts its action through its temporo-menisco-condylar attachments. The extent to which the capsular, i.e. the temporo-menisco-condylar elements affect growth of the condylar cartilage has been only partially determined.Effects of alterations in the tensional level of the capsular unit and transmitted to the condylar cartilage through the periosteal attachemnts of these elements in the condylar process may play some essential role in the regulation of the growth activity under physiologic or experimentally controlled conditions.

Briefly said, following Petrovic's school of thought, increased tension of the lateral pterygoid and, to a lesser degree, of the retrodiscal pad, would induce an increased growth of the condylar cartilage and therefore a supplementary lengthening of the mandible. Further growth of the mandible would be generated by an increased number of dividing prechondroblasts. The experimentally induced stimulation of mitotic activity gives rise to an increase in the vertical thickness of the proliferative layer and subsequently, an increase of the chondroblastic zone later.

The supplementary lengthening of the mandible will reduce the anatomic repositioning of the mandible and, thus, the intensity of the deviation signal. Upon closure, a larger mandible does not have to be displaced so far forward. As a consequence, the increased amount of contractile activity within the pterygoid muscle and capsular frenum diminishes and, therefore, the stimuli for additional growth, decreases.

A histological scenario would be portrayed by a reduction of the hypertrophied layer of the prechondroblastic zone that would trigger the negative feedback to result in a decrease of the mitotic activity in the prechondroblastic zone. According to Petrovic and Stutzman

(1977), these experiments illustrate the mechanism by which the growth of the condylar cartilage adapts itself to meet the functional requirements of a given situation within the craniofacial complex . The mandible will grow until it eventually reaches the dimensions required by new occlusal relationships established by the hyperpropulsor. The signal for further growth increments within the condylar cartilage transmitted by the muscle contractile activity will tend to diminish. The intensity of the stimulation upon the condylar cartilage will become the same as in non-treated animals. Hence, the growth outcome from this point on will no longer be significantly different in both groups (Petrovic and Stutzman, 1977).

Following an initial response, the growth increments will tend to be stable(or tend to stay or tend to remain unchanged) if an appliance is maintained during the entire growth period. If additional growth is desired, the thickness of the appliance must be repeatedly increased. If the appliance is removed before growth is completed, the new occlusal relationship can either be stable (a new intercuspation has been accomplished) or a lack of functional dental engagement between the two arches may be present. In the latter case, a retrusive movement of the mandible will be the immediate response in an attempt to recover the disrupted occlusal intercuspation present previous to to the placement of the appliance. This retrusive movement will tend to decrease the contractile activity of the soft tissue structures involved in the relocation of the mandible. As a result, the intensity of the stimulation signal for the growth activity within the condyle will be less than normal (ie. less than found in non-treated or control animals) and, hence, the growth rate will be lower than expected in non-treated animals from that moment on. The consequence is a relapse with the mandibular position tending to relocate backwards.

These phenomena are an expression of the fact that growth rate and amount in the condylar cartilage is of adaptive nature. It will tend to adjust and respond to the input that

represents the given occlusal relationship and the signal transmitted through the muscle activity (Petrovic et al., 1975; Petrovic and Stutzman, 1977).

Two factors of major importance should be mentioned at this point. First, according to the observations derived from the above experiments (Petrovic et al., 1972, 1975, 1977, 1981) , no genetically predetermined length of the mandible has been detected. Second, adaptation occurs not only in the condylar cartilage but also in other anatomic locations of the mandible. Stimulation of cell division was also noticeable in angular and coronoid locations of the mandibular structure . Increases in periosteal deposition of bone in the posterior border of the ramus have also been extensively reported by Enlow (1979, 1985, 1989). Under the conditions employed in Petrovic's experiments, the overall length of the mandible was significantly greater in animals undergoing mandibular hyperpropulsion than in non-treated animals, but the outcome is the multifactorial integration of all the changes and histophysiological modifications taking place at different loci and acting interdependently (Enlow, 1979, 1990).

The fact that other secondary cartilages, such as coronoyd or angular cartilages of the mandible, seem to respond to functional stimulation of this nature reinforces, rather than contradicts , the accepted interpretation of secondary cartilage as having an adaptive behavior under biomechanical demands. The exclusive and unique role assumed for the secondary cartilage of the condyle by the supporters of the classic theories of mandibular growth as an adaptive process is now acknowledged for other structural components of the mandible. In fact, it has been reiterated by by Enlow (1990) and currently accepted by numerous investigators, that the isolation of condylar response from changes undergone by the mandibular ramus in the common attempt of maintaining good coordination between dental arches and structural harmony within the craniofacial complex, is purely artifitious. The mandible operates as a functional whole, the different parts being responsible for different

degrees of adaptation in the course of the craniofacial development. Another interesting approach has been presented by Moss (1960, 1969) exploring the same underlying concept. He categorizes the different regions within the mandible, and establishes a specific correlation between the bony parts and the surrounding soft tissue structures. Although these two perspectives originated from somewhat different backgrounds, a sense of globality can be inferred from either one of them. Likewise, the understanding of growth process as a sequence of events taking place in an orderly fashion, in a situation of intimate interdependence is noticeable in both analyses.

In a summary, it is pertinent to restate that the coordination of changes in the condylar cartilage as well as coronoid, angular region and ramus as a whole have been surprisingly disregarded . Separation of the mandible into different overly rigid categories serves only the purposes of pseudopedagogic simplification. Such oversimplification retards the development of a mature comprehension of craniofacial biology.

COMPONENTS OF MANDIBULAR GROWTH.SETTING THE STAGE FOR PERIOSTOMY STUDIES

From the information presented thus far in this report, it seems that the two components currently felt to be primarily involved in regulating mandibular growth are the periosteum and the condylar cartilage. The activity of the periosteum has been expressed in terms of aposition and resorption (Enlow, 1981, 1990; Petrovic , 1982). As pointed out by Enlow (1982, 1990) the growth activities reside in the soft tissue of periosteum and endosteum, not in the bone itself. The enclosing osteogenic membranes and other soft tissue structures with attachments to the mandible produce and control bone growth. The periosteal osteogenic activity is essentially tension related (Enlow, 1981, 1982, 1990) because of muscle and connective tissue attachments. The mechanisms regulating bone growth have been reported to be both of systemic nature (hormonal regulation, biochemical

intermediary factors and others) and of local origin, the latter being chiefly represented by muscle contraction and its effects upon the tension of the periosteum (Petrovic and Stutzman, 1972; Petrovic et al., 1975). These mechanisms of local origin bring about apositional or resorptive growth activity and account for the remodeling process.

The question has been formulated (Enlow, 1982) on whether tension, as traditionally believed, specifically induces bone formation. Or, by the same token, whether pressure necessarily carries out (or triggers) resorption. Seemingly, there is no topographic correlation between places of muscle attachment and distribution of resorptive or depository fields (Enlow, 1990). Likewise, the basic mechanism by which muscle contractions induce aposition or resorption remains open to speculative interpretation. Current thought holds that bone growth is the result of a biochemical response to a sequence of bioelectrical potential evoked secondary to muscular activity (McNamara, 1973; Enlow, 1982; Petrovic, 1984).

As discussed earlier, it has been shown that cartilaginous growth in the conbdyle occurs primarily in the proliferative layer. The mechanisms governing this growth, however, remains unclear. Although under the major government of the systemic factors, the responsiveness of secondary cartilage to factors of local origin is believed to be much greater than the one for primary cartilages (Petrovic et al., 1981). The feedback effect, as proposed by Petrovic (Petrovic and Stutzman, 1986) is unique for the mandibular region. The role played by the cartilaginous contribution is understood in terms of a continuous and fine adjustment to achieve a good fit in occlusal relationships as well as in structural balance.

In Petrovic's perspective, growth of the condylar cartilage is regarded as being under permanent exposure to regulatory factors of local origin which allow the structural coordination of different parts within the craniofacial skeleton , coordination between between dental arches and functional integration of other components in the masticatory apparatus.Therefore, local conditions seem to be the requirement "sine qua non" for

systemic factors to act upon condylar cartilage. Using this line of reasoning, Petrovic (1984) has argued that in the absence of normal attachment of the lateral pterygoid, systemic factors such as somatotrophic hormone, would be unable to exert their influence upon condylar growth. Factors from the local environment would enable and enhance the potential influence of systemic factors.

In analyzing the intimate mechanisms of action of biomechanical factors in inducing a response in the condylar region, consideration of the regulation at a cellular level appears indispensable. As it will be shown, discrepant voices have been heard recently as to the apparently arbitrary division between cartilaginous and periosteal components of growth responses.

A precise and more analytical understanding of the sequence of cellular events involved in condylar growth can provide new insights into conventional growth control theories. Furthermore, such an understanding will allow the better stablishment of the biological connections between the periosteal and cartilaginous components in growth regulation in the condylar region.

STATE OF THE ART ABOUT CONDYLAR GROWTH DEFINITION. CURRENT CONTROVERSY

Analyses of mandibular growth which insist on the existence of discrete periosteal or cartilaginous components have been recently criticized for several reasons. Different authors over the last decades (Tammisalo, 1919; Meikle, 1973; Hall, 1978; Thorogood, 1979; Koski and Ronning, 1985) have provided varying degrees of evidence showing that both the periosteal and cartilaginous components have the same cellular origin: the periosteal source of cells, undifferentiated, although most likely committed

Cells of the periosteum cover the mandible and its condylar process (Tammisalo, 1919; Meikle, 1973).The periosteal cell population widely contains relatively undifferentiated cells which have the ability to develop either into the chondrogenic or the osteogenic cell population (Hall, 1978; Thorogood, 1979). Although the chondrogenic cell population of periosteal origin is not completely differentiated, according to some authors they are most likely committed (Hall, 1982; Koski and Ronning, 1985).

In light of merging ideas which suggest the existance of significant commonalities in the cartilaginous and periosteal growth processes, an essential requirement for the eventual understanding of mandibular growth is to define the environmental factors and conditions that allow and promote the manifestation of the commitment of the periosteal cell population, towards the chondrogenic line (Hall 1978, 1982). Attainment of this understanding is made even more complicated because it must be shown how even if the previous commitment is not present, stimulating factors can induce the differentiation of the periosteal cells into a population of the chondrogenic type (Storey, 1972; Meikle, 1973; Simons, 1977; Copray, 1985).

In a similar vein, Petrovic (1982) has observed that " at the boundary between the condylar cartilage and the condylar periosteum, a population of undifferentiated cells is found, that are identified as skeletoblasts. These cells are of mesenchymal origin and can derivate either to prechondroblasts or to preosteoblasts". At this border area, cells that differentiate into prechondroblasts join the mitotic compartment of the condylar cartilage. On the other hand, cells which differentiate into preosteoblasts migrate superficially to incorporate themselves into the periosteal osteogenic population of the condyle. Hence, according to Copray (1985) and to Koski and Ronning (1985), it can be concluded that growth of secondary cartilage in the condylar region depends to an unknown extent on the mitotic activity and differentiation of this periosteal cell population that Petrovic has called

skeletoblasts. The extent of the relative importance of this component of growth of the condylar cartilage in terms of quantity and regulatory nature should be subject of comment on the Discussion section of this paper.

The validity of these premises has been tested by different investigations. Petrovic (Petrovic and Stutzman, 1972; Petrovic , 1982) performed experimental surgical procedures in the growing rat which involved resectioning of the condylar cartilage, superior head of the lateral petrygoid muscle, disc and retrodiscal pad. Based on subsequent histological analysis, Petrovic claims to have proven at a cellular level that if the condylar bone remains intact and the functional mobility of the mandible is preserved, the repair process depends upon the skeletoblastic activity in the lower boundary of the periosteum. Skeletoblasts initially multiply, then migrate and finally cover the resected surface. In the absence of inflammation, recently formed skeletoblasts differentiate into prechondroblasts and subsequently into secondary cartilage, if mandibular mobility is preserved.

Whether the repair process in this situation depends on cells of local origin or not is controversial. However, the concept is supported by the fact that skeletoblasts have the ability to differentiate into prechondroblasts.

Considering the common source for both the chondrogenic and osteogenic population, the separation of periosteal and cartilaginous contributions to mandibular growth (Petrovic, 1982) does not seem meaningful anymore according to some authors (Koski, Ronning and Nakamura, 1985). In addition to that, the mechanism of condylar cartilage growth can not account for the whole growth of the mandible, not even for the condylar region, using a variety of different approaches (Thorogood, 1983; Koski and Ronning, 1985; Enlow, 1990). Likewise, it is conceivable that the periosteal contribution to the condylar cartilage is at least one of major importance. This being the case, control of condylar growth can not be adequately described without understanding the mechanisms involved in

the control of periosteal activity. The latter statement opens the controversy that this study intends to join and, to some extent, elucidate.

It is known that secondary cartilage is responsive to both systemic and local stimulating factors (Petrovic , 1982). The nature of the influence of general regulatory factors seems to be debatable. General factors will be responsible for the control of both the amount, the rate and direction of secondary cartilages by having a direct effect on cell multiplication at the cartilaginous level (Petrovic, 1982).

Thorogood (1979, 1983) holds the idea that general factors can directly influence the mitotic activity at the periosteal level in the same way they affect primary cartilages. Given the correlation between the mitotic activity in the condylar region and the growth effect at the level of the condylar cartilage, it seems tenable to consider the effect of general factors as being both direct and indirect over the condylar growth (Petrovic, 1982).

Local factors, on the other hand, are thought to enable and to enhance mechanisms of general origin as well as to have a direct effect on the direction and the amount of condylar growth (McNamara, 1973; 1975; Petrovic, 1982). Indeed, local factors defining the biomechanical environment are believed to determine the conditions for the differentiation of the periosteal cell population into the chondrogenic line. In addition to that, they will be able to encourage and promote mitotic activity at this level, increasing in this way the mitotic potential of the prechondroblastic layer (intermediate zone) of the condylar cartilage (Petrovic, 1982; Koski and Ronning, 1985). The nature of this biomechanical stimuli of local origin as well as the stepwise regulatory mechanisms that would transform the mechanical signal into a biochemical response have been poorly characterized.

Functional activity, a good example of functional stimulation of local origin, specified as intermittent pressure, has been generally recognized as being necessary for chondrogenesis to take place in the mandibular condyle (Storey, 1972; Meikle, 1973;

Simon, 1977; Copray, 1984). The muscles, the capsule, menisco-temporal frenum and other structures that are attached to the periosteum may determine or contribute to the amount of pressure that is exerted upon the condylar surface (Chong and Evans, 1982) . But, most importantly, the level of tension within the condylar region would be determined by the degree of traction of the soft tissue structures with periosteal attachment in that area (Chong and Evans, 1982). The contractile activity of both superior and inferior heads of the lateral pterygoid along with the rest of the meniscal, capsular and condylar attachments would generate a certain amount of tension upon the periosteum of the condylar region (Petrovic et al., 1985). This mechanical stimulation, in turn, would induce an increment of the mitotic activity stimulation upon the periosteal cell population. Further, it would induce differentiation of skeletoblasts towards the chondrogenic line, with subsequent migration of newly generated prechondroblasts cells and incorporation into the prechondroblastic proliferative layer of the condylar cartilage (Koski and Ronning, 1985; Petrovic et al. 1986). Following this line of thought, changes in the level of tension being exerted upon the condylar periosteum could be responsible for the chondrogenic activity of the prechondroblastic layer of the condylar cartilage.

The notion that periosteal tension levels have a regulatory effect on the condylar cartilage is consistent with the concepts repeatedly expressed by Moss and others (Moss, 1960; Moss and Salentjin, 1969 for example) regarding the workings of the "functional matrix theory": As the mandible is displaced downward and forward as a result of the enlargement and displacement of soft tissues, tension would be generated in the condylar region. This tension would stimulate the periosteal cell population, and bring about a mitotic response of prechondroblastic activity and, subsequently, adaptive condylar growth.

However, this Mossian interpretation , among others, because of the assumptioon it makes has to face severe criticism. Indeed, the assumption is made by Moss (e.g. Moss and

Salentjin, 1969) that facial development brings about differential enlargement of soft tissues involved in systemic functional demands (Functional units) in relationship to the soft tissues specifically related to bony attachments (Skeletal units). Although the reasoning exhibits great consistency with some of the research findings aforementioned in the previous paragraphs , this speculation has not been proven up to date. Furthermore, it can be speculated that if enlargement of soft tissue attachments occurred simultaneously and in quantitatively parallel fashion, relative to the soft tissues involved in mandibular displacement, no tension would be generated in the specific locations of the bony attachments and, therefore, this control mechanism will not be operative.

It can be concluded that further experimental data needs to be provided in order to solve the apparent conceptual contradictions in the interpretation of particular factors involved in mandibular morphogenesis.

EXPERIMENTALLY INDUCED FUNCTIONAL SCENARIO. ADEQUACY OF THE HYPERPROPULSOR DEVICE.

Hyperpropulsion devices have been utilized in a long line of investigations during the last three decades to investigate the adaptative mechanisms of condylar growth and, in a broader perspective, the regulatory factors involved in mandibular development (Charlier, 1969; Petrovic, 1974, 1982; Petrovic and Stutzman, 1972, 1975, 1981, 1990; Elgoyhen, 1972; McNamara, 1973, 1975, 1979; Carlson, 1978, 1980; De Groote, 1984).

Hyperpropulsor appliances induce a forward and downward repositioning of the mandible, producing an anatomical displacement similar to that see in patients undergoing orthopedic correction with functional appliances (McNamara, 1973; Carlson and McNamara, 1978; Petrovic, 1984, 1985). Based on the works of Petrovic, McNamara and Carlson in a number of different investigations involving the use of appliances inducing mandibular protrusion in various types of experimental animals, it is known that this type of displacement

causes a stretching effect of masticatory musculature and, more specifically, of the lateral pterygoid muscle as well as menisco-temporal and capsular structures in the temporo-mandibular joint (Petrovic and Stutzman, 1972, 1986; McNamara, 1972, 1973; Carlson, 1978, 1980). This stretching results in a modification of the contractile activity of the muscles and, subsequently, physiologic adaptation and histological rearrangements of the muscle structure(Petrovic and Stutzman, 1972, 1986; McNamara, 1972, 1973; Carlson, 1978, 1980).These investigators have repeatedly reported an increase in the amount of condylar growth as well as a change in the direction of growth activity in animals undergoing this type of experimentally induced mandibular protrusion .

Changes observed in association with the use of hyperpropulsors have been attributed to an increase in the contractile activity of the lateral pterygoid primarily although the stretching of the capsular attachments has been recognized to contribute to the growth increments in the condylar area (Petrovic et al., 1974, 1977, 1981, 1982, 1985).

An alternative line of interpretation has been presented by McNamara and Carlson (McNamara, 1973, 1975, 1979; Carlson, 1980, 1982) who holds that the growth changes observed under this type of functional environment would be given by a mechanism of neuromuscular adaptation. According to McNamara and Carlson, upon placement of the appliance, the new situation would be detected by neural receptors and transmitted to the Central Nervous System. An eferent response would be then transmitted to the various structures (or components) of the biological environment of the bone, inducing growth increments and remodeling changes in the skeletal components involved. It should be noted that the intimate mechanisms that cause an increase in the growth of the condylar region and, more precisely, an increment in the mitotic activity of the prechondroblastic layer of the condylar cartilage, remains to be properly characterized.

If a hyperpropulsor can be used to induce functional changes that produce an increase in periosteal tension in the condylar region, use of this device may be well suited to test the role played by the periosteum during condylar growth. In fact, experimentally induced mandibular hyperpropulsion could provide an adequate functional environment to further test the role played by the periosteum in the process of mandibular growth under modification of the physiologic conditions, by means of surgical periostomy.

STATEMENT OF THESIS

The periosteum is a major contributing component in the growth of the condylar cartilage under normal conditions. Therefore, the periosteum should play a major role in the mechanisms responsible for the adaptive changes observed in the mandibular condyle under certain local modification of its environment.

Functional appliances have been part of the orthodontic armamentarium during a few decades and their use seems to be justified because they are clinically advantageous when properly used. However, their *modus operandi* has not been sufficiently characterized and it seems obvious that such an understanding of their mechanisms of action would increase their range of effectiveness. The notion that a modification of the tensional levels of the soft-tissue structures with periosteal attachment in the mandiubular periosteum by means of a hyperpropulsion device can induce growth increments, raises the question of the role played by the periosteal membrane in the generation of such increments. The hypothesis that the increase in the tensional levels in the periosteal envelope contributes to the generation and/or regulation of such changes in the growth activity of the condylar process is proposed as a working hypothesis in the present investigation. The effect of biomechanical stimulation by means of a hyperpropulsion device is tested.

In addition to that, several investigations provide various degrees of evidence that the periosteum is a common source for both the cell types involved in growth of the condylar cartilage and the cell types responsible for the apositional and resorptive process. Furthermore, it has been indicated a close interaction in the regulatory mechanisms between the catilaginous and periosteal momponent of growth. Therefore, a surgical periostomy around the neck of the mandibular condyle can affect growth activity of the mandible and its condylar cartilage through a double pathway:

1-Introducing changes in the *status quo* of the biophysical and biomechanical conditions of the periosteum.

2-Affecting the proliferative capability (or potential) of the periosteum, reducing its capacity of nourishing the cellular populations of both the condylar cartilage as well as the osteogenic layer of the bone surface of the mandible; likewise, our procedure can affect the capacity of histodifferntiation of the germinal cells contained in the marginal zone of the periosteum, close to the articular surface of the condylar cartilage.

Finally, the surgical execution of a periostomy around the condylar neck in animals undergoing experimentally induced mandibular protrusion would enable us to better characterize the role played by the periosteum in the growth changes observed with the use of devices that induce anterior repositiooning of the mandible.

The NULL HYPOTHESIS can be expressed as follows:

A periostomy does not modify the adaptive changes that can be observed in the condylar growth in animals undergoing mandibular protrusion.

The ALTERNATIVE HYPOTHESIS can be formulated in the following manner:

The periosteum operates as a major regulating factor of condylar growth under normal conditions. Likewise, when the functional environment is modified as in mandibular protrusion, the tension of the periosteum acts as a major control mechanism of the adaptive changes observed in the condylar growth.

MATERIALS AND METHODS

Animal Model

Forty 21 day-old male rats, Sprague-Dawley strain, were equally divided at random into four groups for experimental analysis.

The composition of the different groups is as follows:

1-In the first group of animals (group I) a bilateral surgical periostomy around the condylar neck was performed and a fixed hyperpropulsor was bonded onto the upper incisors in order to reposition the mandible anteriorly.

2-The second group of animals underwent a bilateral surgical periostomy around the neck of the mandibular condylar process but no appliance was used (group II).

3-In the third group a fixed hyperpropulsor was located and a sham operation was performed bilaterally (group III).

4-.The fourth group of animals served as a control group. They did not undergo any experimental procedure, but a sham operation was performed in all of them.

The extent of the experimental period was of five weeks, beginning the day 0 (21 days of age of the animals) and finishing 35 days later (56 days of age). It was divided into two phases, the first one comprising the first two weeks, moment at which 3 out of the 10 animals included on each group were sacrificed. The second phase extended for the next 21 days, moment of termination of the remaining 7 animals of each group.

Surgical Procedure

The rats were housed with their dams in plastic cages until the experimental procedures were performed, whereafter they were separated from the dams and housed with their littermates.

The animals were brought to a surgical level of anesthesia by the i.m. administration of a mixture of Ketamine hydrochloride 100 mg/ml (KETASET®, AVECO) and xylazine 20 mg/ml (ROMPUN ®, HAVER Labs., USA). The relative proportion of each one of the drugs in the mixture was 0.9 cc of ketamine and 0.1 cc of xylazine. The dose used was 0.12-0.15 cc per 100 gr. of body weight, via intramuscular injection.

An incision was performed following the inferior border of the zygomatic process of the temporal bone. The incision was initiated in the posterior limit of the preauricular region, immediately in front of the ear, and extended anteriorly 7 to 8 mm following as an anatomic reference landmark the inferior border of the zygomatic process of the temporal bone. The dermis and superficial fascia were reflected as well as the parotid gland and duct.

The posterior border of the superficial masseter muscle was exposed along with the posteriosuperior region of the ramus and condylar process up to the temporomandibular joint area.

The masseter was incised transversely and detached from its origin or attachment in the anterioinferior aspect of the zygomatic process to an extension of 5 mm in the area immediately above the condylar neck. Then, both margins of the incision were pushed aside by the use of a surgical microseparator. The exposure so accomplished would allow an adequate surgical approach of the condylar region .

A bilateral circumferential periostomy was performed around the condylar neck, at the approximate level of the notch.

PHOTO II

Mouth holder in place and hyperpropulsor bonded onto the upper incisors.

PHOTO III

First contact of lower incisors with hyperpropulsor in movement of closure.

PHOTO IV

Mandibular protrusion induced by the hyperpropulsion. Comparison betweenphoto IV and V provides an indication of the amount of protrusion, i.e. 2 mm.







The cut was initiated on the medial side outside the capsule, 1mm below the articular surface, and the stripping was extended inferiorly 3-4 mm to the level of the mandibular notch. The initial cutting was performed on the lateral side with the tip of the scalpel. The subsequent stripping was performed with the combined use of a hook like instrument and a periodontal curette.

Similar surgical approach was used on the medial side, except for the fact that incision of the periosteum was started 2 mm below the condylar surface and the extent of the stripping was reduced to 2 mm, to avoid secondary trauma of the lateral pterygoid muscle. The use of the scalpel was avoided on this side for obvious technical reasons.

The effective execution of the surgical procedure in the relatively small surgical field of a Sprague-Dawley 21-day-old rat (the surgical openning was smaller than 10 mm)posed some difficulties. For example, it was impossible to surgically expose the medial side of the condylar region. This restriction limited wholesale stripping of the periosteum to the lateral side of the condylar process. Because of the anatomic position of the lateral pterygoid muscle, especially the inferior limit of its insertion upon the medial suface of the condylar process, this visual limitation made it difficult to effectively strip the medial surface.

The surgical procedure was monitored in all cases under microscopic visualization through use of a disecting microscope, provided with magnifying lenses in a range from 0.63 to 4 units and a 25x eyepiece (JENA LABS., GDR), in order to implement the level of precision of the operation. MIcroscope monitoring of the effectiveness of the cut i.e. depth of the section and extension of the periosteal stripping was performed in the lateral side. It is obvious that this form of control is not feasible on the medial side of the operatory region.

Upon completion of the periostomy, the soft tissues were allowed to return to their normal location, the microseparator removed and the margins of the incision approximated.

The tissues were sutured with 3.0 highly resorbable round catgut and the margins of the skin were approximated and sutured with 3.0 coated regular round catgut.

The surgical procedure so described was performed bilaterally in all animals included in the surgical groups. In the other two groups (control group and group undergoing appliance wearing only) a sham operation was performed consisting on the same surgical approach, but without incising nor stripping the periosteal membrane.

Hyperpropulsor

A hyperpropulsor appliance was used in order to protrude the lower jaw in the young rats corresponding to the first and third groups. Animals from the first group were undergoing mandibular protrusion in combination with periostomy, whereas in the third group only sham surgery was performed.

The design of the device is a modification of the one used by De Groote (1984) and the prescription and indications for fabrication have been conveniently adapted.Two different brands of band material were utilized for the fabrication of the two different sizes that were used during the course of the experiment.The first type of appliance was fabricated from a double-folded single piece of band material of size 0.03x125 and 60 mm of length. The piece was bent over itself as to define a solid unit of 6 mm in length and 3 mm wide. The approximate thickness was 0.8-1 mm, with small variation. At this point, the unit was contoured to be able to adapt in its upper portion to the anterior surface of the upper incisors. The lower portion would define a curved inclined plane that forced a postural advancement of the mandible upon closure. A backing wire mesh was spot-welded to the dental portion of the appliance.

The second type of hyperpropulsor was constructed following the same stepwise procedure described above, from a 0.05x180 size band material and a width of 4.5 mm. The

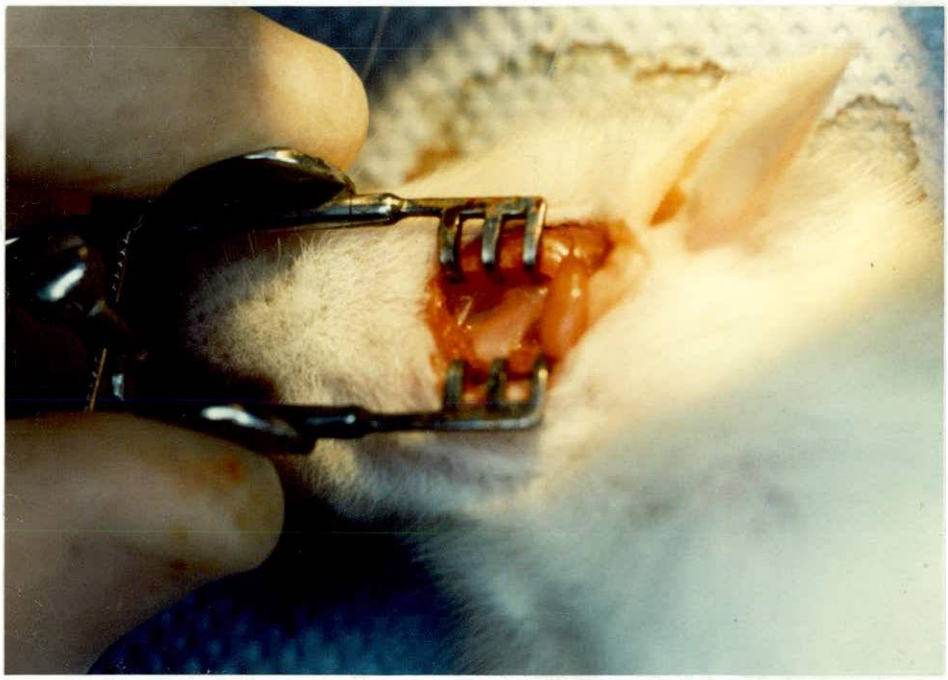
PHOTO V

Surgical approach. Initial cut. Parotid gland reflected posteriorly

PHOTO VI

Microseparator exposing the mandibular condyle and part of the ramus





piece used in this case was 40 mm long, the final appliance exhibiting a length of 8 mm and a thickness of 1-1.2 mm.

The hyperpropulsors of the first and second types (type I and type II) exhibited different characteristics in size, the latter being of a greater thickness, length and width. The rationale for the use of two different appliances is forwardly explained.

The hyperpropulsor type I was utilized in all animals undergoing mandibular protrusion during the first phase of the experimental period (first two weeks). Its size characteristics met the features of the animals at the younger age of 21 days.

At the end of the second week, 3 animals of each group were sacrificed. In the animals undergoing mandibular protrusion during the second phase of the experimental period (the remaining 7 on group I and 7 on group III), the type I hyperpropulsor was removed and the type II was located in its place.

The replacement of the first device for another one of greater size was considered necessary for several reasons. First, it would enable us to account for the eruption of the upper incisor that, upon appliance placement, would not be counteracted by the physiologic wearing of the incisal edge proper of a rodent. Therefore, after removal of the appliance, the incisal edge was remodeled using a fine grain diamond burr. After reduction of the clinical incisal crown of both upper incisors to the level exhibited by the control animals, the second hyperpropulsor was bonded.

Another reason for the use of a second set of appliances was the increase in size to be expected in all animals in the course of two weeks of experimental period, precisely located at the beginning of the growth spurt. Independently of the potential growth stimulation secondary to appliance wearing, the physiologic pubertal growth by itself would be enough to eliminate any substantial protrusive effect induced by the device.

The controversy still persists in the literature regarding the necessary characteristics of design for a hyperpropulsion device as well as its prescription and mode of use to induce an effective increment in the growth rate of the condylar cartilage and mandibular ramus.There are important precedents in the literature reporting either a lack of difference or a decrease *de factu* rather than an increment with the use of hyperpropulsion in animal experimentation. Petrovic (1973) has argued effectively that there exist ideal conditions in which an anterior mandibular repositioning induces a supplementary increment of the growth rate of the mandibule and its condylar cartilage. These ideal conditions can be summarized as follows: a mandibular advancement no greater than 2 mm and a degree of bite opening no greater than 1 milimeter. When the force applied in order to obtain a mandibular advancement is of a great magnitude as well as when the degree of sagittan advancement or vertical opening exceeds the limits considered ideal, the physiologic movements of the mandible are practically impossible to preserve (or are practically eliminated). In fact, the mandible remains immobilized in the position newly acquired through the placement of our appliances. In this circumstances, according to Petrovic (1973), and in light of the negative results obtained by De Groote and others when these norms of design and presciption have not been followed, the expected increments in the rate of growth do not take place. Furthermore, in some cases (De Groote, 1984) it is noticeable a clear decrease of the growth rate of the cartilage. Likewise, the ideal prescription can be established as a wearing time no greater than 18 hours a day, given the fact that, seemingly, no further growth can be achieved with a longer wearing time. Besides, a part-time wearing does not interfere with the feeding habit of the animal. According to several authors (Charlier, 1968; Petrovic, 1973; De Groote, 1984) the full-time wearing prescription is not adviseable since it carries out a negative effect upon the growth activity.

In our study, regarding the dimensional requirements relative to the design of the hyperpropulsion device, every effort was made to fabricate the appliance in such a way as to

obtain a degree of sagittal advancement or repositioning no bigger than 2 mm. and a degree of vertical opening between 1 and 1.5 mm., both in the first and second types of appliances used. Radiographs were taken before and after the placement of the appliance in order to ensure that the degree of protrusion and opening were not excessive. Although, minimal individual variability was always present and we assume that this is also the case for any other study reported in the literature. It becomes a difficult task to obtain exactly the same amount of sagittal and vertical repositioning given individual variations even if we standarize the fabrication of the appliance and use littermates in the construction of our group sample.

Our appliances were constructed in such a way as to provide the same amount of protrusion and vertical opening during the growth process. However, whether the figures of anterior repositioning and bite opening remained the same throughout both phases of the experimental period, is speculative. Upon placement of the second appliance, these parameters were analyzed again and new radiographs were taken to document the new mandibular repositioning. In spite of minimal individual variations, our appliances seemed to provide a mandibular repositioning within values accepted to be effective in inducing stimulation upon condylar growth.

In regard to the prescribed time of wearing, our appliances were worn on a full-time basis (24 hours) provided they were fixed, as opposed to the removable type advocated by Petrovic and others, where the wearing time can be regulated. In this respect, we did not meet the requirements considered ideal for the prescription of use of the device. The reasons that led us to use a fixed hyperpropulsor are forwardly explained.

The design of the hyperpropulsor described following the model of Petrovic, Stutuzman et al., consists in a removable appliance including a dental cap to be loosely fitted on the upper incisors of the experimental animal to which a sophisticated wire frame is attached. A neck strap and a system of elastic ligation will ultimately help to relate the different

parts and allow the appliance to stay in place. Besides, an acrylic collar must be fitted around the neck to prevent the animal from removing the appliance by the use of its limbs. This type of appliance has been used by the mentioned authors in a large series of studies and has proven to be effective and not to compromise the viability of the rats in any way.

The main rationale for the use of this appliance is twofold. Firstable, the appliance does not interfere with the feeding habits of the animal, allowing the rat to eat normally during nightime. The second reason,as it has been stated before, is that no further groiwth seems to be obtained beyond an appliance wearing period of 18 hours, according to Petrovic's results(1977, 1985, 1986, 1987). Further advantages of this aplliance are that no difference is found in the weight curve between the experimental and control animals at the end of the experiment. This allows for a perfect extrapolation of the results regarding growth increments and bone size.

However, two major inconvenients arise for us with the use of this appliance. The first one is regarding the complex anatomy of the device that complicates enormously the proper fabrication. In addition to that, because of its design, the appliance must be replaced periodically in a growing animal, and a new one must be constructed and fitted in place. The animals must be supervised every hour because of the risk of dislocation of the dental unit. These circumstances make the use of this appliance simply impossible in the absence of a very specifically oriented laboratory and well trained animal care personnel.

Due to the referred limitations, an alternative appliance was considered. Our design followed the basic principles proposed by De groote (1984) and posteriorly used by Easton and Carlsson (1989) as to the fabrication of a fixed appliance bonded to the upper incisors. One of the major criticisms articulated against the appliance used by De Groote was its excessive bulkiness that resulted in an inhibitory effect of the growth of the condylar cartilage, although his results showed the opposite trend in his pilot study, using the same appliance.

We did emphasize the importance of the dimensional characteristics of our appliance during the design and fabrication, as well as ensured the amount of sagittal repositioning and vertical opening accomplished with our appliance. The rats adpted very quickly to it and the feeding behaviour did not change significantly, other than a preference of the rat for the soft chow. It is to bear in mind that the appliance had to be changed after two weeks in order to maintain the desired characteristics of the mandibular repositioning. The weight curve did not exhibit any statistically significant difference relative to the controls.

The appliance was bonded onto both upper incisors with a paste-paste composite orthodontic material following a standarized technique. The mouth was maintained in a maximal open position and the tongue kept out of the oral cavity by the use of a customized adjustable holder. This device, consisting in a single-looped oval wire-made frame (to be known as " Mouth Holder of Oyen"), was located immediately behind both upper and lower incisors pressing the tongue outwards , downwards and laterally and displacing the upper lip upwards in order to expose the complete incisal crown. It was vertically open as to achieve the desired amount of oral aperture.

The upper incisors and surrounding structures were dried out with an air source and the teeth were etched with phosphoric acid at 37% during one minute.They were rinsed and dried again before the application of the resin. The two paste composite was then mixed and applied to the wire mesh backing of the appliance. An orthodontic bracket holder (or positioner) was used to locate the device with accuracy.

A lateral cephalogram was taken before and after the placement of the propulsor in order to ensure the amount of protrusion and vertical opening that was achieved.

Recovery and follow up

After the surgical procedure and/or placement of the appliance, all the animals exhibited a normal recovery. The animals were separated from their mothers and housed in

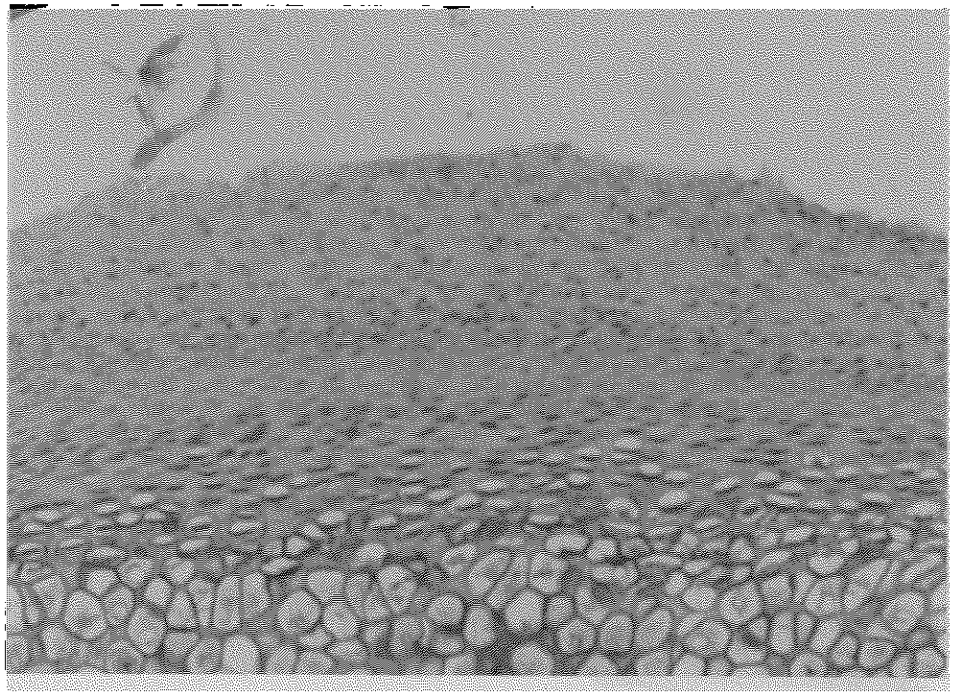
PHOTO VII

A 16x microphotograph of a section corresponding to the hyperpropulsion-only group. Note the good representation of the different layers and the increasein the dimensional features of the cartilage. Cells are loosely arranged.

PHOTO VIII

A 16x microphotograph of a specimen corresponding to the surgery-only group.Note the compact arrangement of hyperchromatic prechondroblasts in the growth zone. The functional zone has practically disappeared and the hypertrophic chondroblasts are well arranged.





cages as weaned littermates. Some previous studies emphasize the presence of an important inflammatory infiltrate of the medular component, during the inmediate postsurgical period (Koski and Ronning, 1974, 1985) To prevent against the likelihood of problems associated with inflamation or infection, a phase of antibiotic therapy was initiated .

The antibiotic coverage was provided by the administration of Penicillin V potassium (BEEPEN ®, BEECHAM Labs) mixed in the water at a dose of 25 mg/ml of solution, equivalent to 40.000 units.

Likewise, during the postoperatory period, a combination of hard and soft rat chow was provided. All animals resumed normal eating habits during the first two days post-operation. The feeding behavior did not exhibit significant changes in the experimental animals as compared to the control group, other than a preference of the former for the powdered rat chow. Both fluids and solid diet were administered "ad libitum" in all groups.

All animals from both the control and experimental groups were weighted at the beginning of the study (day 0, preoperation) and every three days until the day of the sacrifice. A weight chart was elaborated for every group. No weight differences were found among the different groups.

First Phase-Sacrifice at fifteen days

As it has been mentioned previously, three animals of each group were sacrificed at the end of the first phase of the experimental period, after two weeks.

In previous periostomy studies, it has been shown that an inflammatory reaction follows the surgical procedure and persists during the first five days of the observation period. In fact,the firs reaction after the periostomy has been reported to be the expected on in a soft tissue wound, with presence of a moderate to intense inflammatory infiltrate in the area. The marrow tissue exhibits a severe infiltration of red cells with cellular displacement of all other series, that appear significantly decreased or absent.

The surgically induced discontinuity of the periosteum, according to the majority of authors, undergoes a healing process as soon as on the third to fifth day postoperationem.The periosteum is found to have healed by the ninth or tenth day after surgery. Osteogenic activity is already noticeable at this time.

So, in an attempt to determine the comparative changes observed in all groups a few days after the periosteum had presumably healed, three animals of each group were sacrificed at the day 15 of the experimental period.

The growth activity at this point is supposedly affected by the periostomy and, under the hypothesis object of this study, the degree of affectation would decrease as the normal osteogenic activity is being restablished after healing.

Therefore, the purpose for the sacrifice of the first set of animals was to run a comparative analysis between the different groups at a moment where the effects of surgery were undoubtly to be found.

Moreover, the specimens obtained at this point would enable not only a comparison between groups, but also an intragroup analysis of the effects observed in animals undergoing the same procedure at different stages of the experiment.

As it appears obvious, the main advantage of this step would be to evaluate differences in the surgical groups during the disrupted-periosteum phase and the healed-periosteum phase.

End of the experimental period and Sacrifice

Previous to the termination, 14 or 34 days of the experimental period, the animals received an intraperitoneal injection of Colchicine, a mitotic inhibitor, 1 mg/1000 gm. of body weight in 10% saline solution, six hours prior to the sacrifice.

In order to obtain hard-copy records of the skull prior to the histological preparation, the animals were placed in a specially designed cephalostat, a modification of the one described by Palmer (1978), to insure reproducible object film and source film distances. Radiographs were taken utilizing the Broadbent Bolton cephalometer (Series 1K550, Bolton Fund, Cleveland, Ohio) and standard dental occlusal film Kodak, Df46, Rochester, N. Y.). Each animal was oriented both in norma lateralis and in norma dorsalis. Following the methodology developed by Hans (1981), the animals were placed in a vertical position with the midsagittal plane oriented parallel to and 3 cm from the film, so that lateral cephalograms could be taken. Maintaining the same position of the animal and using another X-ray tube perpendicular to the first one, a dorso-ventral projection was obtained for every one of the animals, the film being in close contact to the ventral surface of the head. A set-up of the cephalometer was used of 90Kv, 15 mA and an exposure time of 1 1/2 seconds for the norma lateralis and 80 Kv, 10 mA and an exposure time of 2 seconds for the norma dorsalis.

Several cephalometric landmarks were determined and measurements were taken in order to allow eventual assessment of the macroscopic differential changes that might have occurred in mandibular growth among the groups.The heads were skinned, cut sagittally into haves, fixed in Zenker's solution and decalcified in 5% formic acid.

Histological Analysis

Histological evaluation of the specimens corresponding to the different groups was carried out the following way. Since the histological analyses included morphometric features as well as the dimensional study of the different layers of the condylar cartilage, it was considered justified to select comparable sections in regard of the level of the cut and the depth of the condyle from which the section had been extracted.

Ten histological sections were selected for each animal in order to analyze the different parameters object of study in sections clearly representative of the intermediate

thickness of the mandibular condyle, showing a good representation of the different layers of the condylar cartilage.

Study of each section included the analysis of thickness (vertical height) of every one of the layers of the condylar cartilage as well as the measurement of the total height of the cartilage, from the superior limit of the proliferative zone to the inferior limit of the hypertrophic layer, where the line of erosion begins.

The lineal dimensional analysis of the thickness of the different layers was conducted through the incorporation of a calibrated ruler into one of the ocular eyepieces. This additional dispositive (ruler) is mobile, permitting its orientation to be moved within the microscopic field. In each section the total height of the condylar cartilage plus the individual thickness of each of the layers which comprise the condylar cartilage were measured using a calibrated ruler in one of the ocular eyepieces in the following manner. Under low magnification, i.e. objective lenses less than 10x, the ruler was rotated and the slide moved horizontally until the ruler was aligned with the long axis of the condyle and approximately bisected the maximum transverse dimension of the condyle in two equal halves, as visualized in a magnifying screen placed onto the microscope. Once the ruler was positioned in such a way as to superimpose the longitudinal axis of the condyle, the vertical height corresponding to the different layers was performed, under the magnifying lense of 16x, keeping the same orientation of the histological preparation and the ruler. The values were registered and posteriorly translated into μm by a conversion factor previously known.

In a similar manner, a reticular grid in the other eyepiece of the microscope was used to measure areas of the respective layers of the condylar cartilage. These measurements provided some indication about the amount of growth activity in each one of the layers

The lense of 16x was also used for the surface analysis and the orientation of the specimen and lense in order to conduct the measurement following the longitudinal axis of

the condyle, was obtained following the same procedure explained for the linear measurements.

The surface analysis tried to measure the area contained in every one of the layers of the condylar cartilage within a predetermined measuring unit. In order to perform this measurement, the histological sections were adequately oriented by projecting them on the screen located onto the microscope, and using the lenses of low magnification of 8x and 10x and the 10x eyepiece. The measuring unit was determined as a rectangle of one square unit in the base of the grid and vertically rpojected, along the longitudinal axis, throughout the entire vertical height of the cartilage.

The calculation of the surface area was conducted by multiplying the height of the layer as measured with the ruler previously, by the base of the rectangle, whose value in μm was previously calculated. The values were then expressed in square unitsof μ2.

The calculation can be mathematically expressed as follows:

Vertical height x Base of the rectangle= Area contained in the measuring unit

The histomorphometry was considered completed upon measurement of the surface as well as the vertical height of the different layers of the condylar cartilage.

The second stage in the data collection process consisted on the analysis of the cellularity present in every one of the of the layers of the condylar cartilage. therefore, to gain further quantitative information about cellular activity, total counts of the cells enclosed within the reticular grid in each layer at 16x and 40x objective lens magnifications were carried out.

As it has been mentioned previously in this section of Materials and Methods, a mitostatic was injected to the animals six hours prior to the sacrifice. The use of a mitostatic like the Colchicine arrests the cellular cycle in the metaphase. The methodological criteria followed to identify the cells in this phase of the division cycle consisted in the identification of the features that are characteristic of the metaphase. Namely, the presence of an intensely

stained chromatinic mass of irregular form and, in a second place, the loss of the limits of the nuclear membrane.

The microscopic identification of these features in the prolifertive layer of the condylar cartilage is not an easy task, specially if we consider the prechondroblastic mitotic activity that takes place in this layer.

Hence, the mitotic count conveys a margin of error that is difficult to minimize. Likewise, the inclusion in the mitotic count of all the cells exhibiting features characteristics of the division process (that is, including metaphase and telephase) was also attempted, without a satisfactory level of accuracy.

For the above reasons, both the mitotic index and the total mitotic count were discarded, given the considerable difficulty and, consequently, inaccuracy of distinguishing a homogeneous population of cells with clearly noticeable features indicating that the cell division process was taking place.

Therefore, the alternative of total counts was chosen. The histological sections were analyzed both with a 16x and a 40x magnifying lense. The histological preparations were oriented in such a way as to align the grid with the longitudinal axis of the condyle. The same measuring unit utilized for the surface analysis was used for the analysis of cellularity of the different layers. The counts were performed alternatively under the 16x lense and the 40x lense several times until the counts of a given section would coincide within less than a 5% of difference (or until figures with less than 5% differnce were obtained). The total number of cells were counted for every one of the layers.

Given the indistinct limits between layers, in most cases, within the condylar cartilage, a precise criteria was set up in order to differentiate cells in the borderline areas as belonging to one compartiment or the other. And so, in the limit between the Proliferative and the Functional chondroblast layers, cells exhibiting some degree of hypertrophy, decreasing staining intensity of afinity, showing some activity of matrix production and forming a

somewhat looser arrangement, were considered to be part of the functional chondroblast zone. At the boundary between the the Functional Chondroblast zone and the Hypertrophic zone, cells adopting a more vertical orientation with their long axis perpendicular to the articular surface, exhibiting an unstained citoplasmis margin, reorganizing themselves into a compact arrangement with important reduction of intercellular matrix, were considered to be part of the hypertrophic layer.

The limiting area between the articular surface and the prechondroblastic zone did not offer difficulties in terms of cell identification, nor did it the inferior border of the hypertrophic layer, in its contact (or in its limit) with the erosion line. The evaluation of the cellularity corresponding to the different layers was then reported, following the mentioned procedures.

Adequate correlations between the values obtained from the different groups relative to the histomorphology or cellularity were performed as it is presented in the following section of this paper.

RESULTS

In the present investigation 40 21-day-old rats were equally divided in four groups for experimental analysis. As shown in the table below, in the first group (group I) a bilateral circumferential periostomy was surgically performed around the neck of the condyle and a fixed hyperpropulsor device was bonded onto the upper incisors of the rats in order to achieve an anterior repositioning of the mandible. In the second group (group II) only the surgical procedure was done. In the third group (group III)only the hyperpropulsor device was placed and the last group (group IV) served as control.

The groups were named according to the following criteria:

Combined treatment : GROUP I

Surgery only : GROUP II

Hyperpropulsion only: GROUP III

Control : GROUP IV

At the end of the second week, 3 animals on each group were sacrificed. At the end of the fifth week, remaining 7 animals each group were sacrificed. Following sacrifice each animal was prepared and analyzed histolgically, as described in the Methods section. Specific features considered in these analyses were individual thickness (vertical height) of the three fundamental layers of the condylar cartilage; i.e. growth zone, functional chondroblast and hypertrophic zone; also considered were cell counts in each of these zones. Results of these analyses, organized on the basis of the two-week and five-week phases are summarized below. The mean values for the measurements obtained for the different groups are provided with the analysis carried out for each layer.s for the dimensional analysis is presented. The surface analysis was simply calculated as a function of the linear measurements, provided that the standarized measuring unit had a constant base and the height of the surface to be calculated was given by the height of the layer in every case. Therefore, the surface values

are not represented, although their calculation was performed, showing, as expected, absolute consistency with the linear results. The numerical representation provided in this section regarding the dimensional parameters pertains, for this reason , to linear values of the height of every layer. From the linear values, the area values can be easily calculated multipliyng the height of each layer (linear measurements) by the base of the grid utilized as a measuring unit that was of 180 μ. The product is the area of that particular layer contained in the measuring unit. So, it is important to bear in mind that from the linear measurements, a perfect extrapolation can be made with the results of the surface analysis.

A ONE-WAY ANOVA was performed for the comparison among the measurements of the different groups during phase one. A similar statistical analysis was carried out for the phase two measurements. Two-way analyses of variance were carried out in order to asses the interaction of the various procedures performed in the different groups with the different phases of the experimental period.

FIRST EXPERIMENTAL PHASE-DIMENSIONAL ANALYSIS

A.-Growth Zone (ML1)

At the end of the first phase of the experimental period (two weeks), the analysis of the dimensional values in the growth zone, carried out in the different group provided the following information.The parametric one-way ANOVA statistical analysis is presented in Table IA-1.The mean values of the measurements are provided in Table IA-2 and Fig IA-1 and Fig IA-2. The Kruskal-Wallis test is presented in Table IA-3.

No statistically significant difference among the four groups during the first phase was found. Given the distribution of the values for this particular parameter, a non-parametric test was considered necessary in order to confirm our first estimation. Application of a non-parametric one-way ANOVA Kruskal-Wallis test subsequently confirmed the lack of significance noted among the four groups.

Variable ML1 · MEASUREMENTS-GROWTH ZONE

By Variable GROUP PHASE ONE

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	37.6689	12.5563	5.4803	.0297
Within Groups	7	16.0383	2.2912		
Total	10	53.7073			

TABLE IA-1. One-way Analysis of Variance of the values obtained for the vertical height of the growth zone . Give the distribution of the data for this parameter (∂=2.7062 as shown in Table IA-2) a non-parametric test was considered necessary to confirm the analysis presented in this table.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	2	9.0500	.3536	.2500	5.8734	To	12.2266
Grp 2	3	12.6000	.6557	.3786	10.9710	To	14.2290
Grp 3	3	7.7667	2.7062	1.5624	1.0441	To	14.4892
Grp 4	3	9.3333	.4509	.2603	8.2132	To	10.4535
Total	11	9.7455	2.3175	.6987	8.1885	To	11.3024

Table IA-2. Representation of the mean measurements of the vertical height of the growth zone in the different groups. The means calculated for each group are presented. Note that the count (second column) for group I (combined treatment) is N=2, indicating that one case was excluded because of extremely discordant values.

Kruskal-Wallis 1-way ANOVA

ML1 MEASUREMENTS-GROWTH ZONE

by GROUP PHASE ONE

Mean Rank	Cases	
4.25	2	GROUP = 1
10.00	3	GROUP = 2
3.67	3	GROUP = 3
5.50	3	GROUP = 4
	--	
	11	Total

			Corrected for Ties	
CASES	Chi-Square	Significance	Chi-Square	Significance
11	6.4735	.0907	6.5030	.0895

Table IA-3. Non-parametric Kruskal-Wallis one-way Analysis of Variance conducted for the mean values of the vertical height of the growth zone, during the first phase of the experiment. This test confirmed the lack of statistically significant difference among the groups (p=0.0895).

FIG. 1A-1 GROWTH ZONE MEASUREMENTS PHASE I VS PHASE II

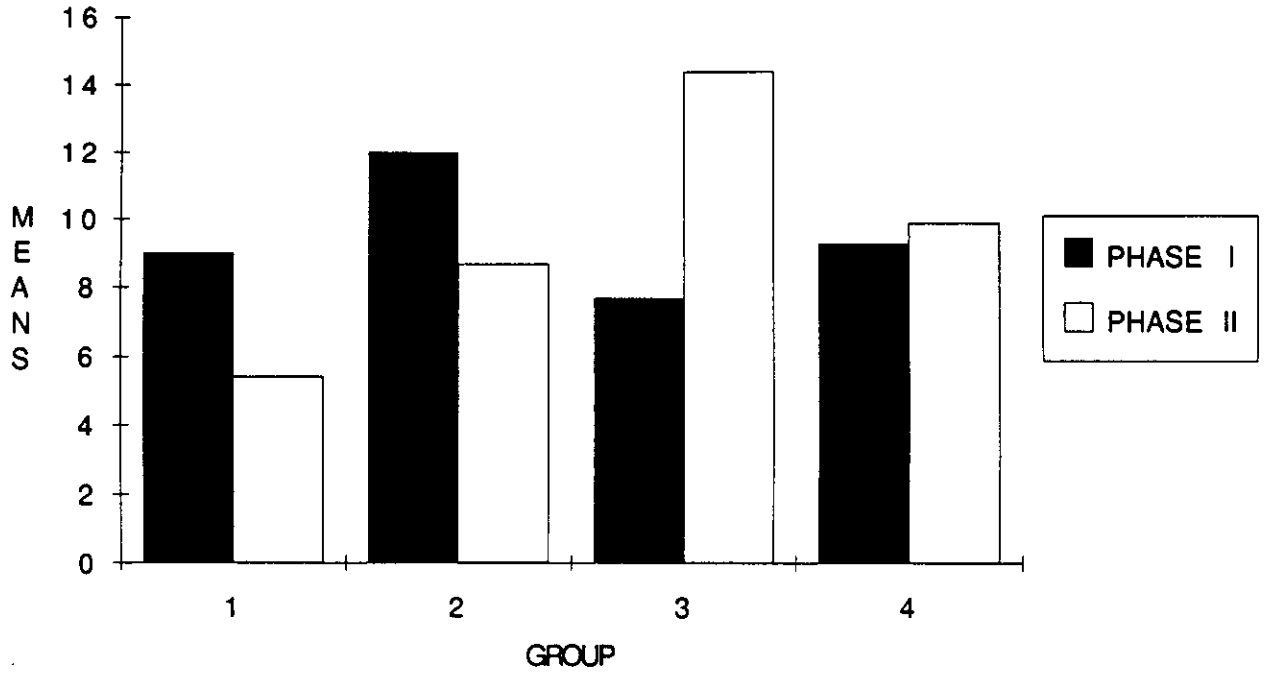
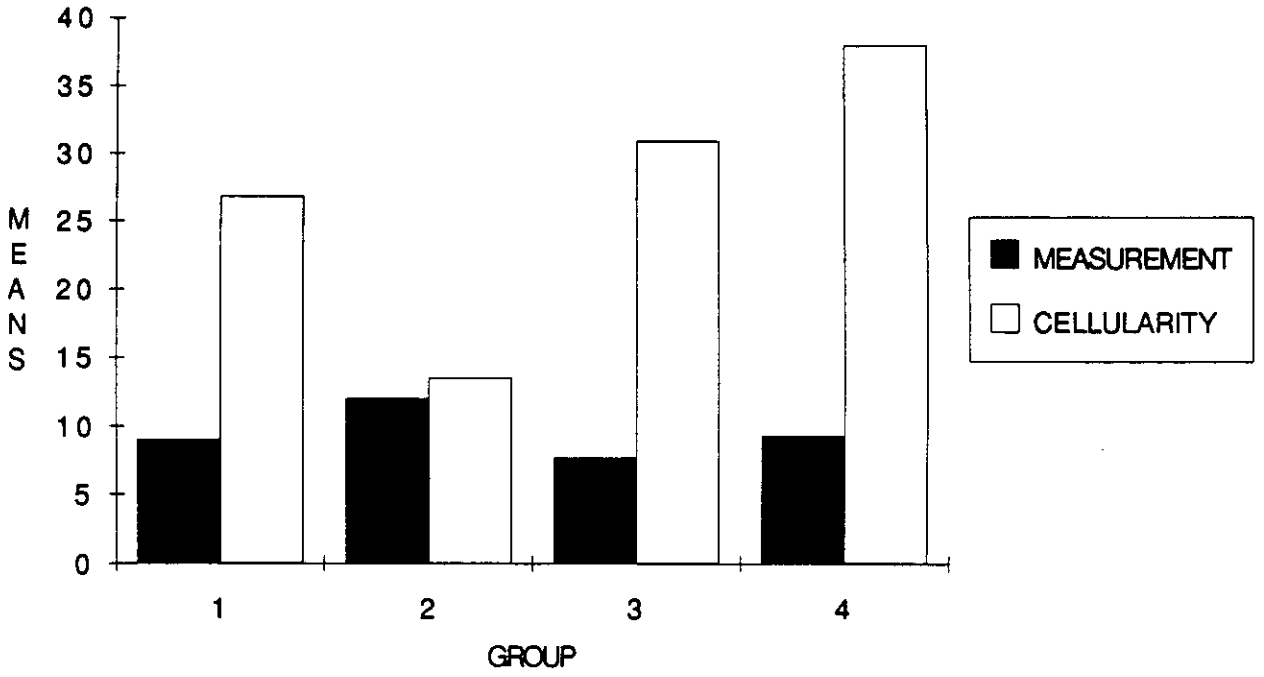


FIG.IA-2 GROWTH ZONE-MEASUREMENTS VS CELLULARITY. PHASE I



B.-Functional Chondroblast Zone

The statistical analyses and mean values are provided in Tables IB-1 and IB-2 and mean values are represented in Figures IB-1 and IB-2.

In the underlying layer, at the end of the first phase, the order of the different groups according to the values obtained for the dimensional analysis of the functional chondroblast zone is the same as the one observed for the growth zone, although in the former the statistical significance is extremely high for the comparison between groups.

The surgical groups exhibit values slightly greater than controls. The group of combined therapy has values relatively close to the group of surgery alone (Group II) and the control group (group IV), but the group undergoing hyperpropulsion alone (group III)presents values significantly smaller than any of the others.

C.-Hypertrophic Zone

The statistical analyses and mean values are provided in Tables IC-1 and IC-2 and mean values are represented in Figures IC-1 and IC-2.

No statistically significant difference among the mean values of the four groups was found for the hypertrophic layer in the first phase of the experiment. Paiwise comparisons were done using the Scheffe's procedure and the Tukey-HSD test, confirming that no two groups were different at a statistically acceptable level.

D.-Total measurements

The statistical analyses and mean values are provided in Tables ID-1 and ID-2 and mean values are represented in Figures ID-1 and ID-2.

Given the relative consistency of the order in which the different groups appear in the ranks of each one of layers, the values of total measurements observed for both the linear measurements and the surface analysis follow, obviously, the same pattern or trend previously reported for the individual zones.

Variable ML2 MEASUREMENTS-FUNCTIONAL ZONE

By variable GROUP PHASE ONE

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob
Between Groups	3	93.8847	31.2949	17.9684	.0011
Within Groups	7	12.1917	1.7417		
Total	10	106.0764			

Table IB-1. One way Analysis of Variance of the mean values obtained for the vertical height of the functional chondroblast zone. Note the statistical significance of the test (p=0.0011).

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	2	16.0500	.0707	.0500	15.4147	To	16.6853
Grp 2	3	14.9667	1.6503	.9528	10.8672	To	19.0662
Grp 3	3	8.4000	1.5100	.8718	4.6490	To	12.1510
Grp 4	3	13.3000	1.0440	.6028	10.7065	To	15.8935
Total	11	12.9182	3.2569	.9820	10.7301	To	15.1062

Table IB-2. Representation of the mean measurements of the vertical height of the functional chondroblast zone in the different groups. The means calculated for each group are presented.

FIG. IB-1 FUNCTIONAL ZONE-MEASUREMENTS PHASE I VS PHASE II

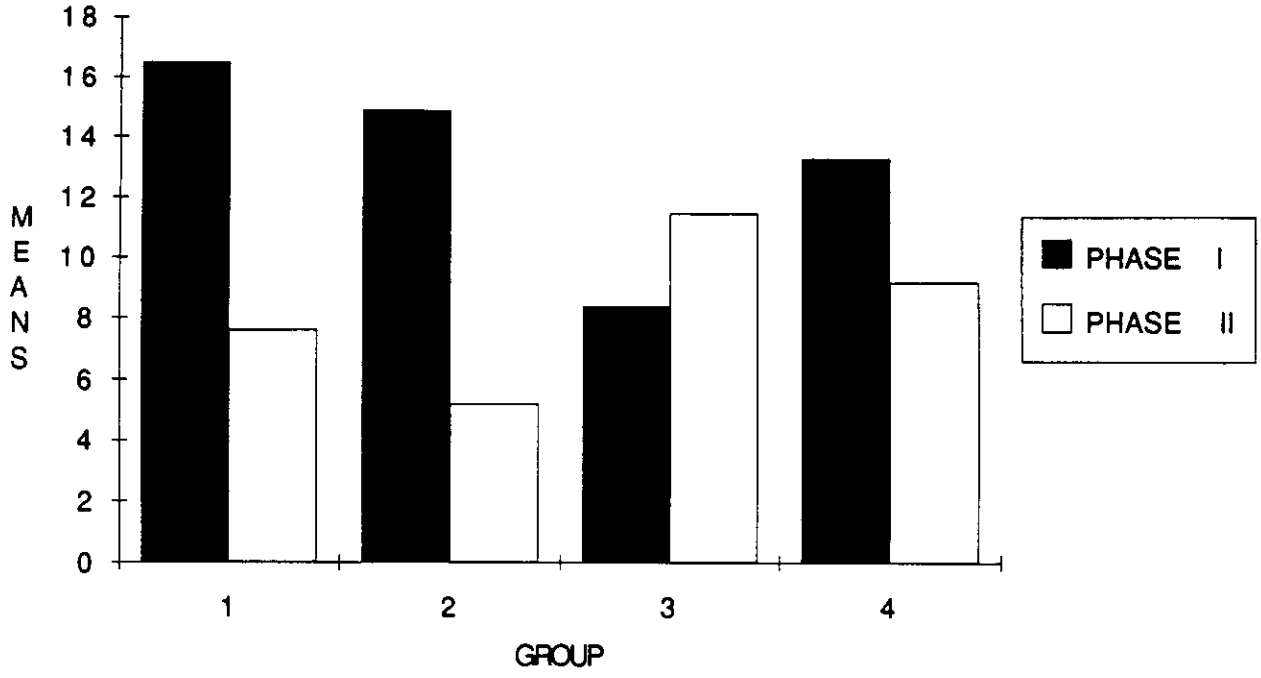
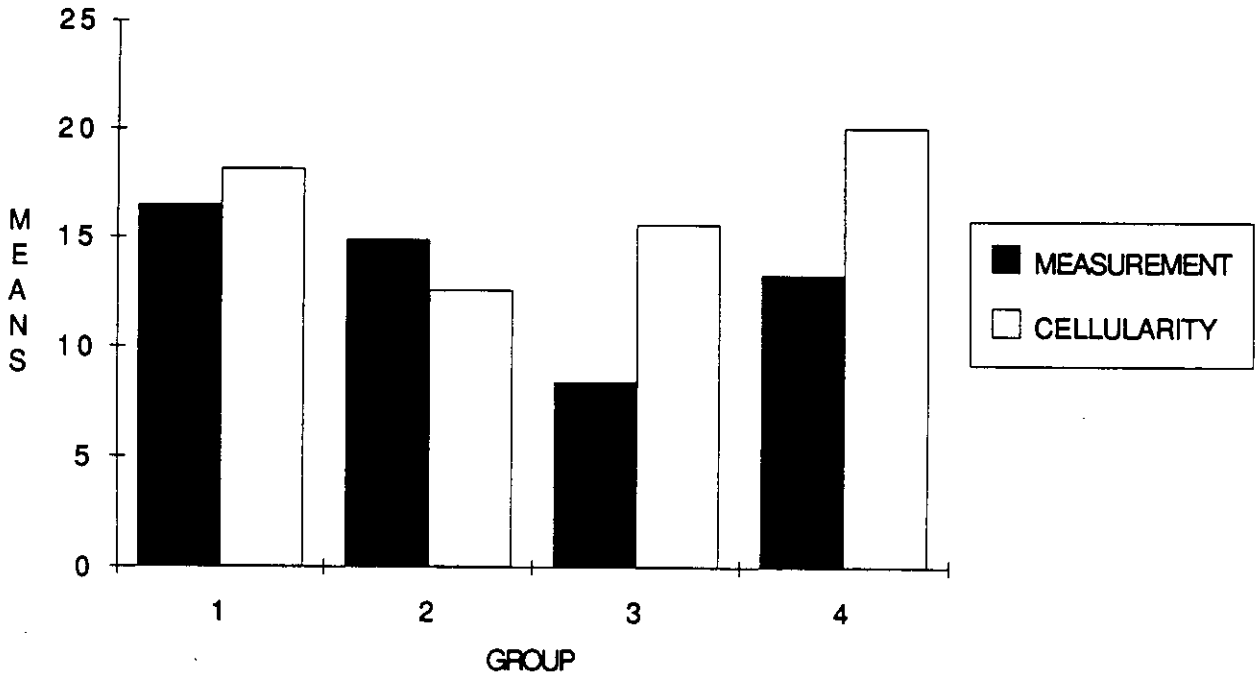


FIG. IB-2 FUNCTIONAL ZONE-MEASUREMENTS VS CELLULARITY. PHASE I



Variable ML3 MEASUREMENTS-HYPERTR. ZONE

By Variable GROUP PHASE ONE

Analysis of Variance

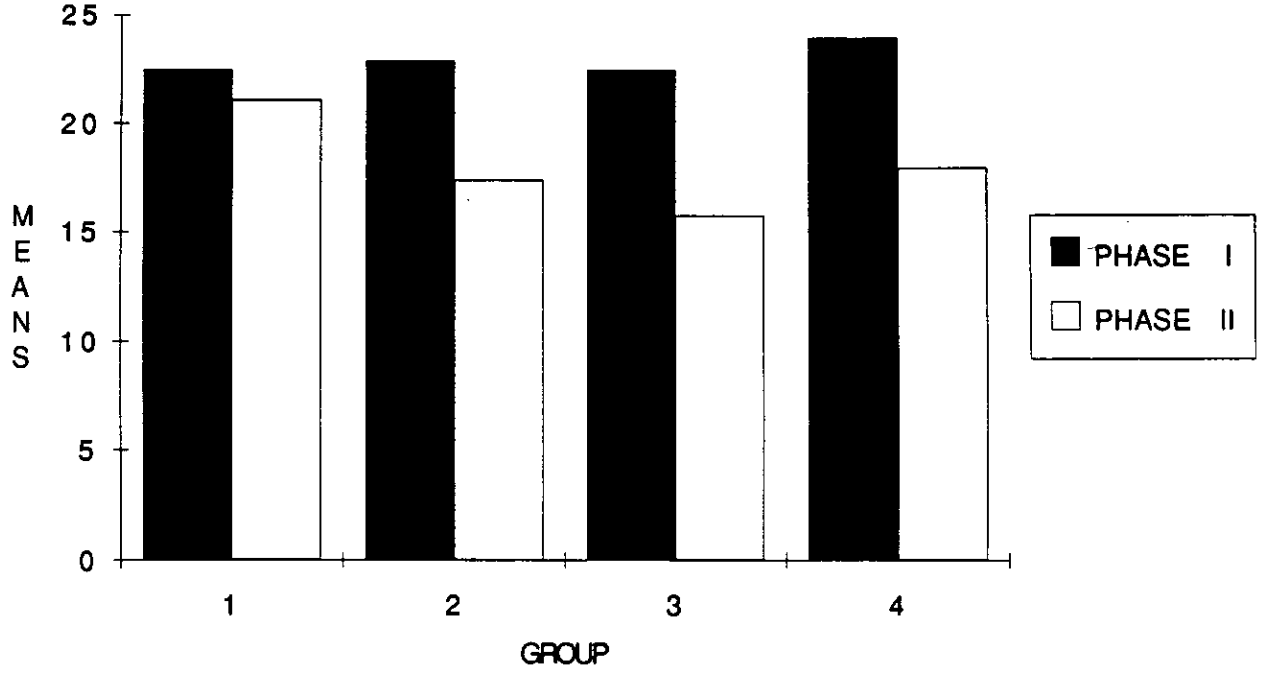
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	3.9489	1.3163	.0980	.9586
Within Groups	7	94.0383	13.4340		
Total	10	97.9873			

Table IC-1. One-way Analysis of Variance of the mean values obtained for the vertical height of the hypertrophic zone as expressed in the table below. The p value (F Probability) for the analysis of the differences among the frour groups is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	2	22.5500	4.4548	3.1500	-17.4745	To	62.5745
Grp 2	3	22.9333	2.9143	1.6826	15.6937	To	30.1730
Grp 3	3	22.5667	5.2003	3.0024	9.6482	To	35.4851
Grp 4	3	24.0000	1.2490	.7211	20.8973	To	27.1027
Total	11	23.0545	3.1303	.9438	20.9516	To	25.1575

Table IC-2. Representation of the mean measurements of the vertical height of the htypertrophic zone in the different groups. The means calculated for each group are presented.

FIG. IC-1 HYPERTROPHIC ZONE-MEASUREMENTS PHASE I VS PHASE II



Variable MTOTAL MEASUREMENTS-TOTAL

By Variable GROUP PHASE ONE

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	224.9380	74.9793	4.6301	.0436
Within Groups	7	113.3583	16.1940		
Total	10	338.2964			

Table ID-1. One-way Analysis of Variance for the mean values of the total vertical height of the cartilage in the different groups. The analysis for the differences among the four groups (p=0.04) is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean
Grp 1	2	47.6500	4.7376	3.3500	5.0842 To 90.2158
Grp 2	3	50.5000	4.9153	2.8378	38.2896 To 62.7104
Grp 3	3	38.7333	4.1861	2.4168	28.3344 To 49.1323
Grp 4	3	46.6333	1.9425	1.1215	41.8078 To 51.4588
Total	11	45.7182	5.8163	1.7537	41.8107 To 49.625

Table ID-2. Representation of the mean values of the total vertical height of the cartilage. The means obtained for the different groups are presented.

FIG. IC-2 HYPERTROPHIC ZONE-MEASUREMENT VS CELLULARITY. PHASE I

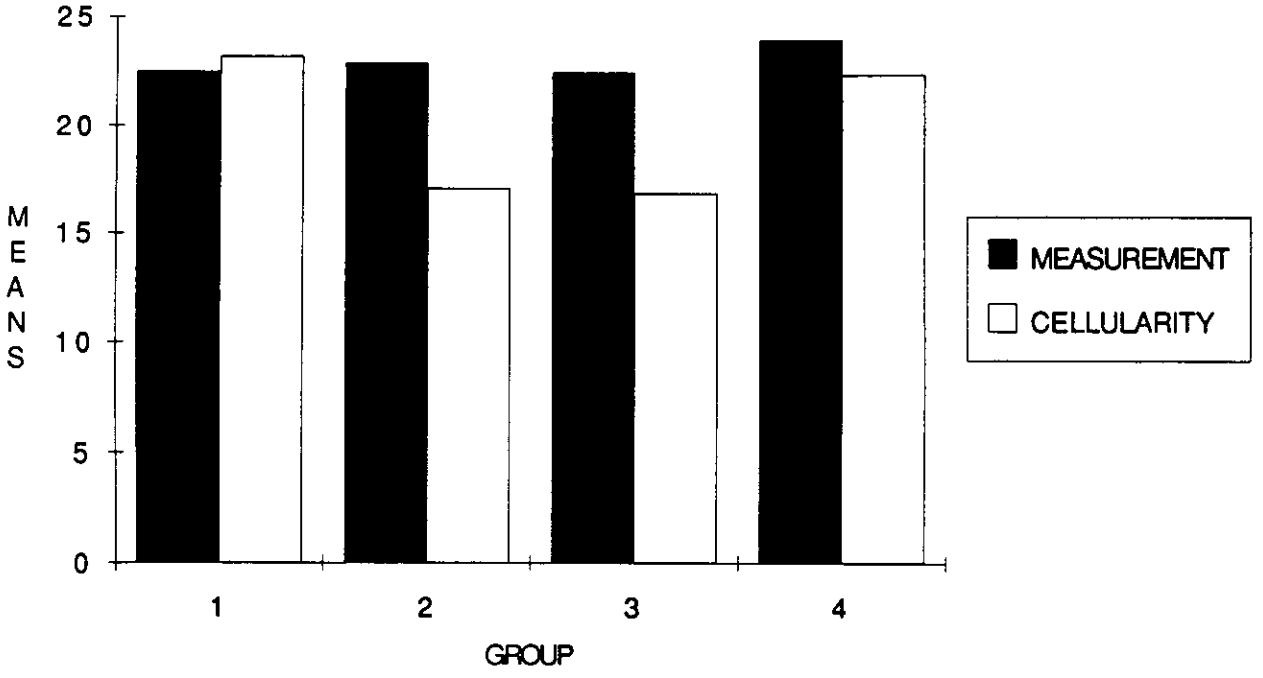


FIG. ID-1 MEASUREMENTS-TOTAL CARTILAGE. PHASE I VS PHASE II

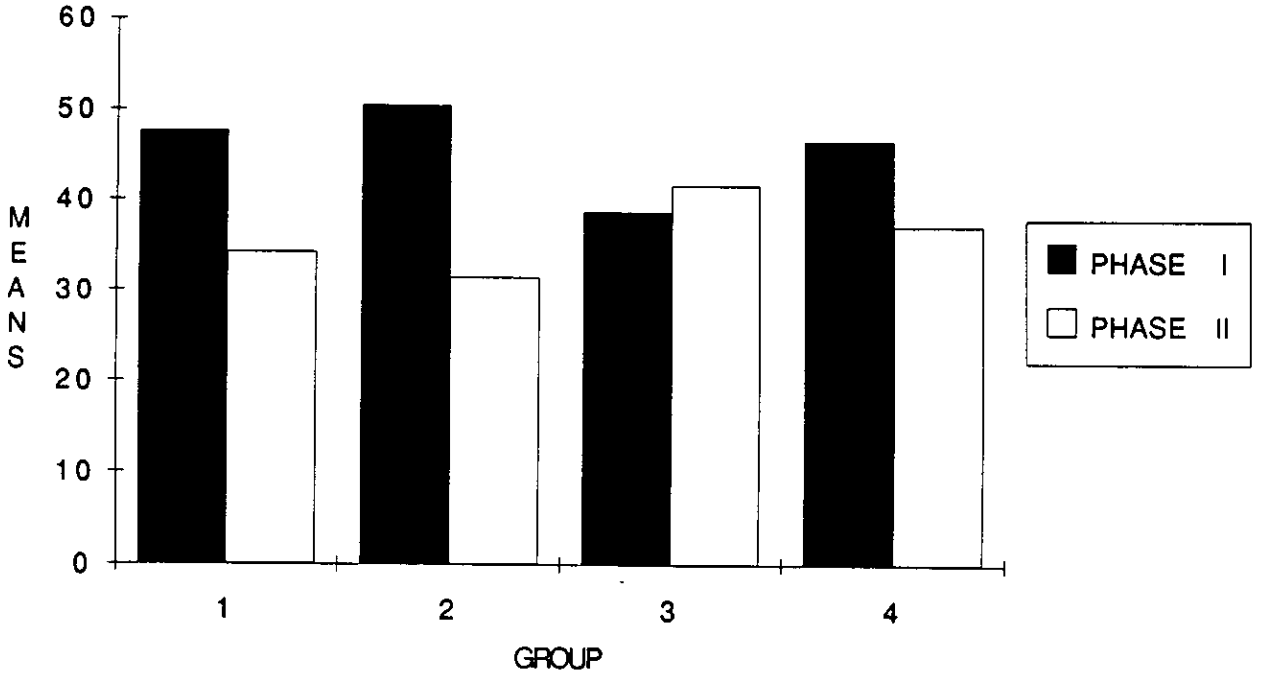
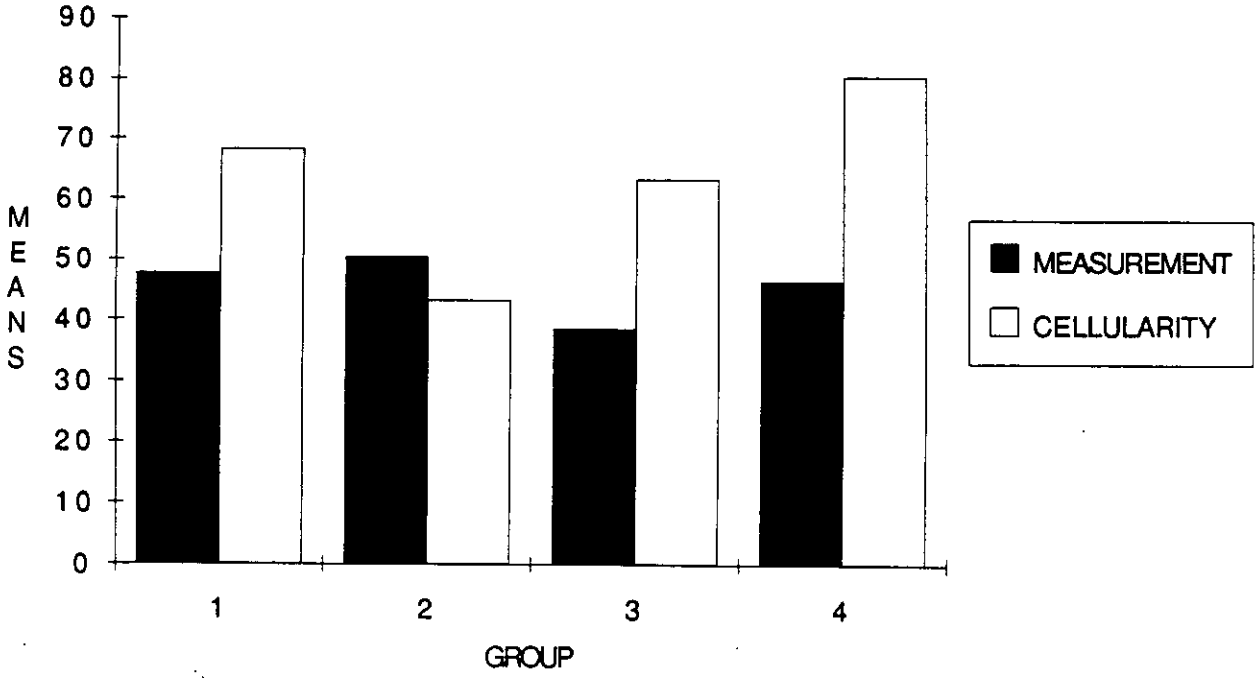


FIG.ID-2 TOTAL CARTILAGE-MEASUREMENT VS CELLULARITY. PHASE I



A primacy of the surgical groups can be observed, that is more evident for the group of surgery alone (group II), exhibiting the greatest values. The hyperpropulsion group (III)exhibits values significantly smaller than the rest. The pairwise comparison indicates a statistically significant differences between group II and the remainimg groups. Other pairwise comparisons between groups do not provide the same level of significance and can not be considered equally important.

FIRST EXPERIMENTAL PHASE-CELLULARITY ANALYSIS

Total counts of cells contained in each of the layers of the condylar cartilage in the different groups were performed at the end of the first phase of the experimental period, and the values obtained are presented as follows.

A.-Growth Zone

The statistical analyses and mean values are provided in Tables IIA-1 and IIA-2 and mean values are represented in Figures IIA-1 and IA-2.

The groups can be ordered from least to most cellularity levels as follows: II, I, III and IV. The proliferative layer of the condylar cartilage in the control group shows the greatest dimensional values in this moment of the experimental period. In fact, the control group seems to have maintained its cellularity levels better than any other one. The surgical groups appear with values significantly lower than the rest statistically, occupying the last positions; group II (surgery only) shows the greatest decrease in the cellular population of the mitotic compartiment relative to the other groups. The hyperpropulsion group (III) keeps its cellularity levels above the surgical groups but its values are smaller than the values of the control group.

The analysis provides evidence that these results are extremely significant, from the statistical point of view. Pairwise comparisons carried out following both the Scheffe's and the Tukey-HSD tests indicate that major differences can be found between the control group

Variable CL1 CELLULARITY-GROWTH ZONE

By Variable GROUP PHASE ONE

Analysis of Variance

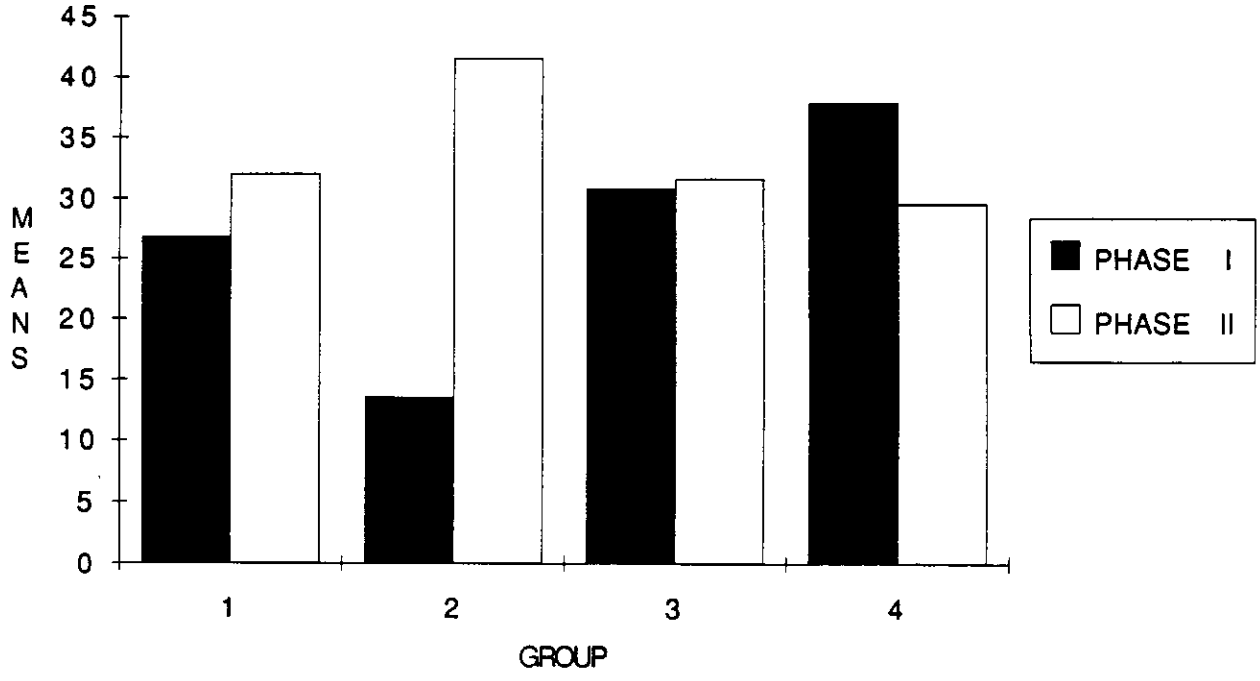
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	954.8606	318.2869	28.4016	.0003
Within Groups	7	78.4467	11.2067		
Total	10	1033.3073			

Table IIA-1. One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the growth zone. Note that the test is statistically significant (p=0.0003).

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean
Grp 1	2	26.8000	2.9698	2.1000	.1170 To 53.4830
Grp 2	3	13.5000	.8660	.5000	11.3486 To 15.6514
Grp 3	3	30.9333	2.6102	1.5070	24.4491 To 37.4176
Grp 4	3	38.0000	5.2202	3.0139	25.0323 To 50.9677
Total	11	27.3545	10.1652	3.0649	20.5255 To 34.1836

Table IIA-2. Representation of the mean values for the cell counts carried out in the growth zone in the different groups. The means calculated for each group are presented.

FIG. IIA-1 GROWTH ZONE-CELLULARITY PHASE I VS PHASE II



(group IV) and the surgery only group(II), the latter presenting very depressed figures of its cell counts of the growth zone.

B.-Functional Chondroblast Zone

The statistical analyses and mean values are provided in Tables IIB-1 and IIB-2 and mean values are represented in Figures IIB-1 and IB-2.

The order of the groups according to the values, from the lowest to the greatest ,obtained in the cellularity counts performed in the functional chondroblast layer is similar to that reported earlier for the cellularity of the growth zone: II, I, III and IV.

The control group has great values, followed by the group of combined treatment(group I). The group of hyperpropulsion (III) and the group of surgery alone (II) occupy the lowest positions.

Differences among groups are statistically significant (p=0.002) for the overall analysis of all groups in this category. The pairwise comparisons show, likewise, statiscally significant differences for the comparisons of the control group with groups III and II. The differences with the group of combined treatment are not as great, but still significant statistically. Differnces between groups III and II are not significant.

C.-Hypertrophic Chondroblast Zone

The statistical analyses and mean values are provided in Tables IIC-1 and IIC-2 and mean values are represented in Figures IIC-1 and IC-2.

No statistically significant difference among the mean values of the four groups was found for the hypertrophic layer in the first phase of the experiment. Given the distribution of the values for this particular parameter, a non-parametric test was considered necessary in order to confirm our first estimation. Application of a non-parametric one-way ANOVA Kruskal-Wallis test subsequently confirmed that no two groups are significantly different at an acceptable level of statistical analysis.

Variable CL2 CELLULARITY-FUNCTIONAL ZONE

By Variable GROUP PHASE ONE

Analysis of Variance

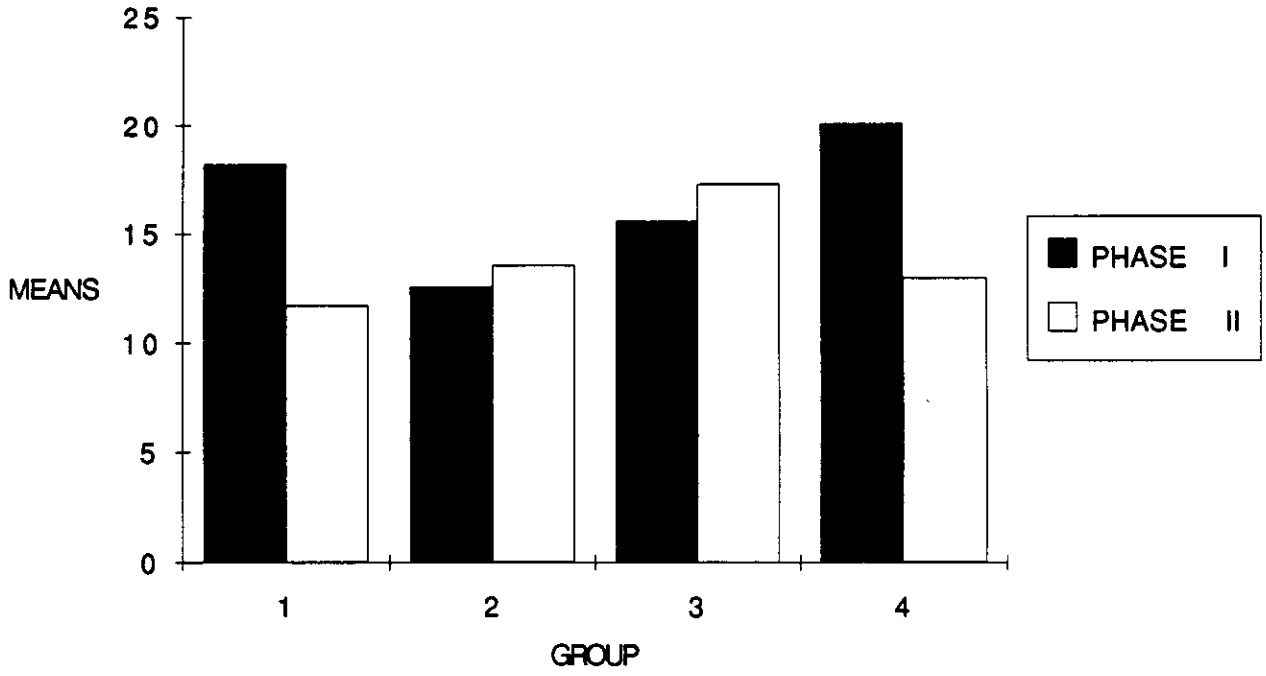
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	91.8958	30.6319	13.7863	.0025
Within Groups	7	15.5533	2.2219		
Total	10	107.4491			

Table IIB-1. One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the functional chondroblast zone. The test is statistically significant since the p value (F Probability) for the analysis of the differences is p=0.0025.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	2	18.2000	1.5556	1.1000	4.2232	To	32.1768
Grp 2	3	12.6667	2.0207	1.1667	7.6468	To	17.6865
Grp 3	3	15.6000	.6000	.3464	14.1095	To	17.0905
Grp 4	3	20.1333	1.4572	.8413	16.5135	To	23.7532
Total	11	16.5091	3.2779	.9883	14.3069	To	18.7112

Table IIB-2. Representation of the mean values obtained for the cell counts carried out in the functional chondroblast zone. The means calculated for each group are presented.

FIG. IIB-1 FUNCTIONAL ZONE-CELLULARITY PHASE I VS PHASE II



Variable CL3 CELLULARITY-HYPERTR. ZONE

By Variable GROUP PHASE ONE

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	89.8205	29.9402	3.9795	.0603
Within Groups	7	52.6650	7.5236		
Total	10	142.4855			

Table IIC-1. One-way Analysis of Variance of the mean values obtained for the cell counts carried out in the hypertrophic zone. The significance for the analysis of the differences among the four groups is provided (p=0.063).

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean
Grp 1	2	23.2500	.4950	.3500	18.8028 To 27.6972
Grp 2	3	17.1667	1.6862	.9735	12.9778 To 21.3555
Grp 3	3	16.9667	.8963	.5175	14.7401 To 19.1932
Grp 4	3	22.4667	4.7501	2.7425	10.6667 To 34.2667
Total	11	19.6636	3.7747	1.1381	17.1277 To 22.1995

Table IIC-2. Representation of the mean values obtained for the cell counts carried out in the hypertrophic zone. The means calculated for each group are presented. Given the distribution of the sample in this parameter (∂=4.7501), a non-parametric test was considered necessary to confirm the result of this statistical analysis.

Kruskal-Wallis 1-way ANOVA

CL3 CELLULARITY-HYPERTR. ZONE

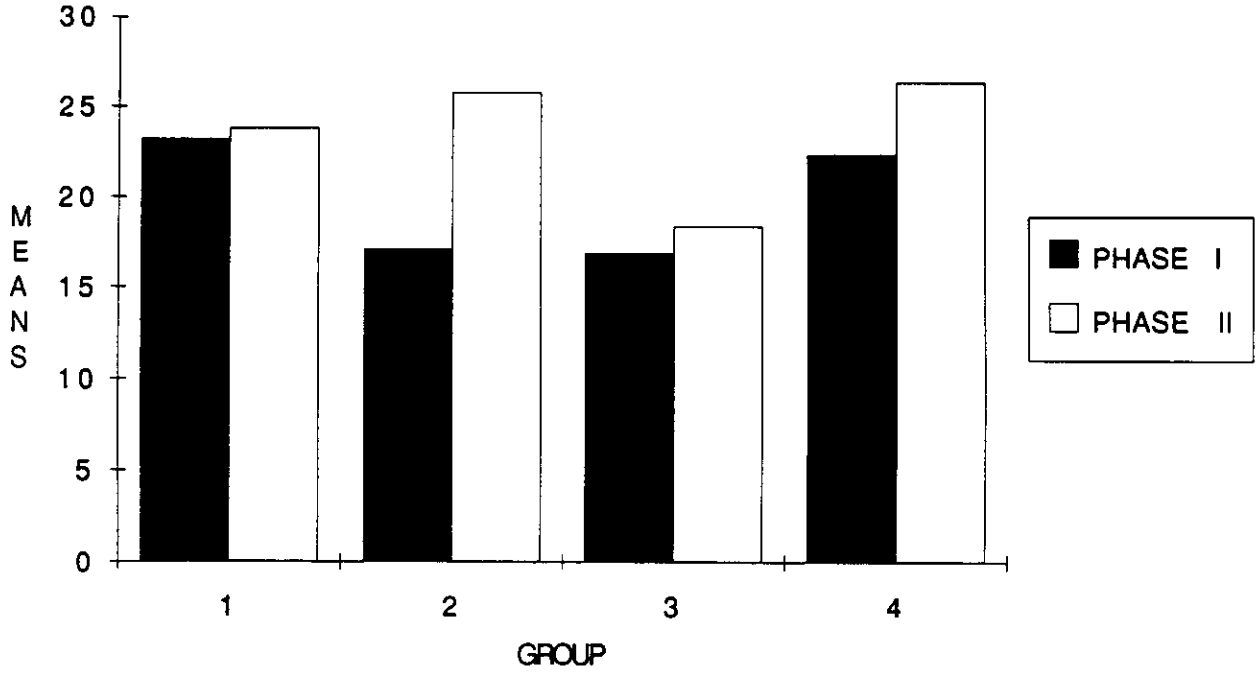
by GROUP PHASE ONE

Mean Rank	Cases	
9.50	2	GROUP = 1
3.50	3	GROUP = 2
4.17	3	GROUP = 3
8.00	3	GROUP = 4
	--	
	11	Total

			Corrected for Ties	
CASES	Chi-Square	Significance	Chi-Square	Significance
11	5.9394	.1146	5.9665	.1133

Table IIC-3. Non-parametric Kruskal-Wallis one-way Analysis of Variance conducted for the mean values of the cell counts of the hypertrophic zone, during the first phase of the experiment. This test confirmed the lack of statistically significant difference among the groups (p=0.1133).

FIG. IIC-1 HYPERTROPHIC ZONE-CELLULARITY PHASE I VS PHASE II



D.-Total Cellularity Analysis

The statistical analyses and mean values are provided in Tables IID-1 and IID-2 and mean values are represented in Figures IID-1 and ID-2.

The results of the comparative evaluation of the total cellular counts performed in the different groups showed to be statistically significant at a very high level ($p<0.00001$).

In the overall analysis, the control group exhibited the highest cell count values, immediately followed by the group I. Groups III and II presented smaller values, as it was the case in the analysis of cellularity of the functional chondroblast zone.

Pairwise comparisons indicate of a very high level of significance in the differences between the control group and any other one, especially when compared to group II. The latter, presented also significant differences when compared to the group III and group I

SECOND EXPERIMENTAL PHASE

At the end of the experimental period (five weeks) the remaining animals, 7 on each group, were sacrificed and studied. The analysis conducted on each one of the 10 histological sections selected for each specimen was the same as detailed for the first experimental phase. The results obtained are forwardly presented.

PHASE II-DIMENSIONAL ANALYSIS

A.-Growth Zone

The statistical analyses and mean values are provided in Tables IIIA-1and IIIA-2 and mean values are represented in Figures IIIA-1 and IA-1.

The dimensional evaluation of the specimens corresponding to the different groups in relationship to the growth zone reflects that the hyperpropulsor-only group (III) maintains greatest values than the rest of the groups, in contrast to what happened at the end of the first experimental phase, what is consistent with the results previously reported in the literature by various authors.

Variable CTOTAL CELULARITY-TOTAL

By Variable GROUP PHASE ONE

Analysis of Variance

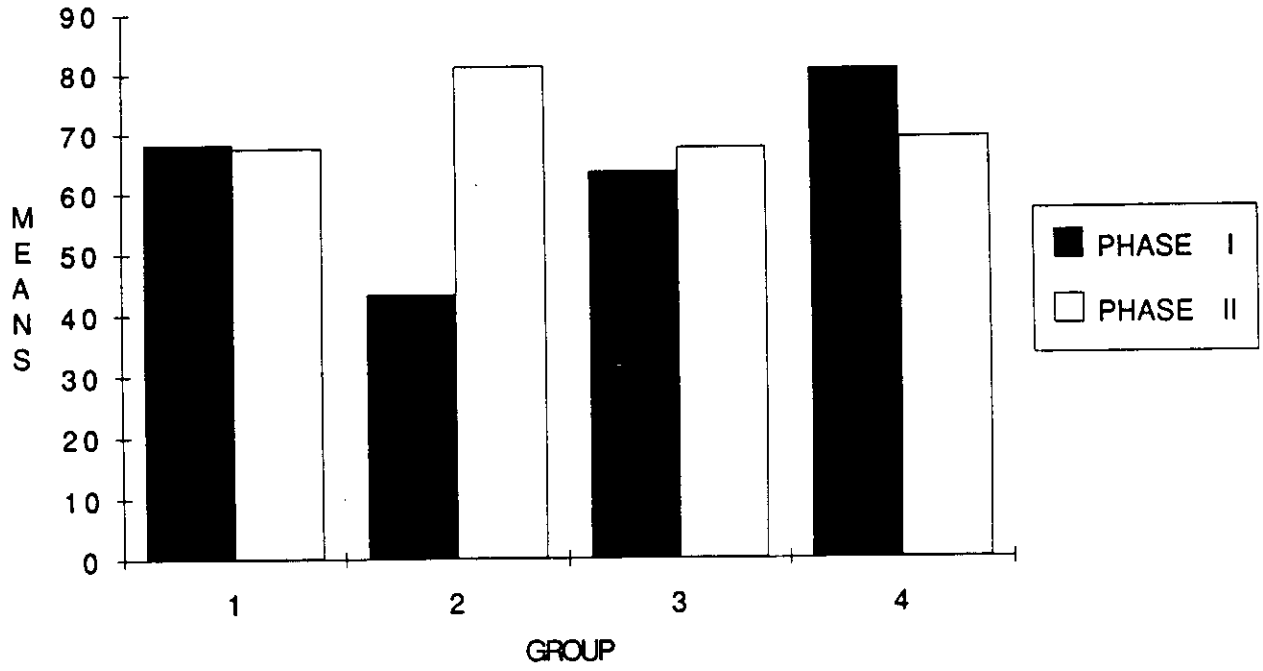
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	2142.4302	714.1434	59.5320	.0000
Within Groups	7	83.9717	11.9960		
Total	10	2226.4018			

Table IID-1. One-way Analysis of Variance for the mean values of the total cell counts. The analysis of the differnces among the four groups is provided. Note the significance of the test ($p<0.00001$).

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	2	68.2500	.9192	.6500	59.9910	To	76.5090
Grp 2	3	43.3333	4.5654	2.6359	31.9920	To	54.6747
Grp 3	3	63.5000	3.4655	2.0008	54.8910	To	72.1090
Grp 4	3	80.6000	2.9513	1.7039	73.2686	To	87.9314
Total	11	63.5273	14.9211	4.4989	53.5031	To	73.5514

Table IID-2. Representation of the mean values obtained ffor the total cell counts. The means calculated for the four different groups are presented.

FIG. IID-1 CELLULARITY-TOTAL CARTILAGE. PHASE I VS PHASE II



The surgical groups have the smallest values, without significant differences between the two of them. The control group presents values above the surgical groups but smaller than the group of hyperpropulsion (group III)

The overall analysis proves to be very highly significant ($p<0.00001$). The paiwise comparisons are extremely significant from the statistical point of view for comparisons between the group III and any other group.

Comparisons established between the control group and the surgical groups prove to be statistically significant as well, but at a lower level.

Given the distribution of the values, a non-parametric test was considered necessary in order to confirm the provisional conclusions provided by the conventional one-way ANOVA. The kruskal-Wallis one way ANOVA test was conducted and the difference resulted to be very highly significant also in this analysis ($p=0.0003$ as shown in Table IIIA-3).

B.-Functional Chondroblast Zone

The statistical analyses and mean values are provided in Tables IIIB-1and IIIB-2 and mean values are represented in Figures IIIB-1 and IB-1.

The hyperpropulsion group exhibited the greatest values for the measurement performed in the functional chondroblast zone, as it was the case for the proliferative layer, the differences with the other three groups being statistically significant. The least-to-most ranking of the groups is as follows: II, I, IV, III. Group II holds the last position presenting the lowest values. Groups I and IV occupy positions in between groups III and II, their differences not being statistically different. The overall analysis was statistically significant in the differences among groups, all groups considered.

Variable ML1 MEASUREMENTS-GROWTH ZONE

By Variable GROUP PHASE TWO

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	270.8088	90.2696	17.6771	.0000
Within Groups	23	117.4512	5.1066		
Total	26	388.2600			

Table IIIA-1. One-way Analysis of Variance of the mean measurements of the vertical height of the growth zone. Note that the test is statistically significant but the distribution of the data in this particular parameter (see Table IIIA-2 shown below) required a non-parametric test to confirm the results of the test presented in this Table.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	5.4167	1.3014	.5313	4.0509	To	6.7824
Grp 2	7	8.7857	3.4290	1.2960	5.6144	To	11.9570
Grp 3	7	14.4000	1.8788	.7101	12.6624	To	16.1376
Grp 4	7	9.9714	1.6958	.6409	8.4031	To	11.5398
Total	27	9.8000	3.8643	.7437	8.2713	To	11.3287

Table IIIA-2. Representation of the mean measurements of the vertical height of the growth zone at the end of the second phase. Note that the count (second column) for group I is N=6, indicating that one case was excluded because of extremely discordant values. Given the distribution of the data (∂=3.4290) in this particular parameter, a non parametric test was done, in order to confirm the significance of the analysis above.

Kruskal-Wallis 1-way ANOVA

ML1 MEASUREMENTS-GROWTH ZONE

by GROUP PHASE TWO

Mean Rank	Cases	
4.50	6	GROUP = 1
11.93	7	GROUP = 2
23.43	7	GROUP = 3
14.79	7	GROUP = 4
	--	
	27	Total

			Corrected for Ties	
CASES	Chi-Square	Significance	Chi-Square	Significance
27	19.0181	.0003	19.0472	.0003

Table IIIA-3. Non-parametric Kruskal-Wallis one-way Analysis of Variance conducted for the mean values of the vertical height of the growth zone, during the second phase of the experiment. This test confirmed the statistically significant difference among the groups (p=0.0003).

FIG. IIIA-1 GROWTH ZONE-MEASUREMENTS VS CELLULARITY. PHASE II

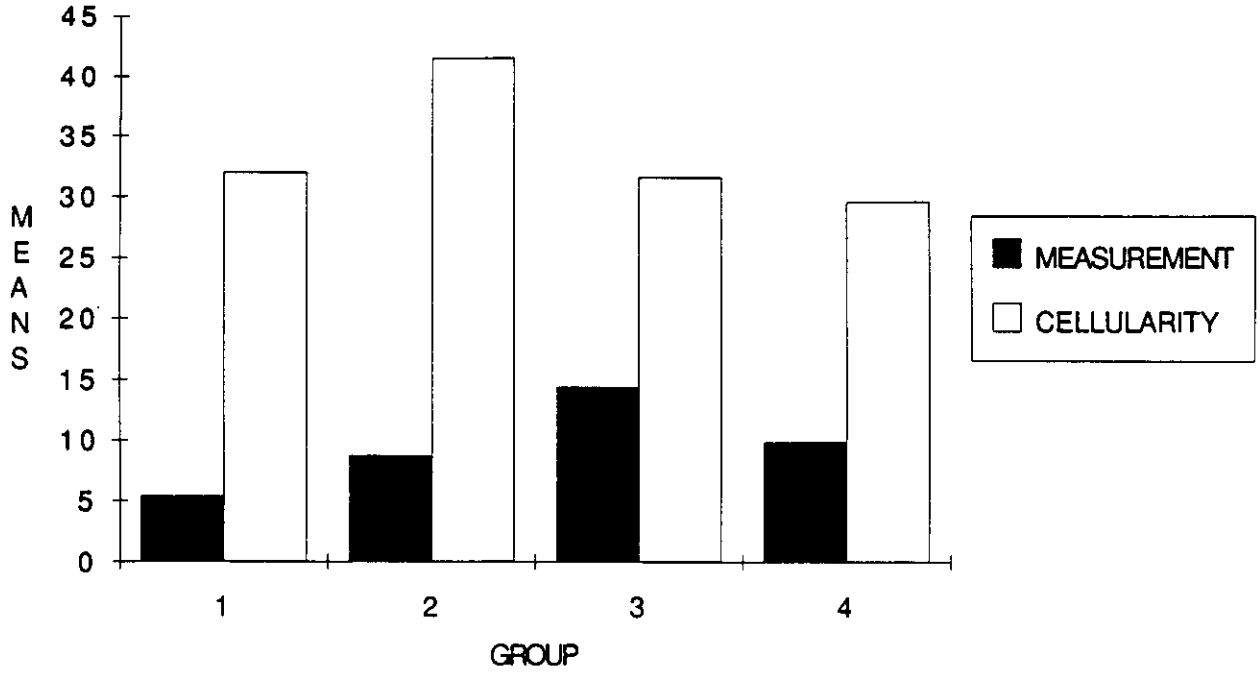
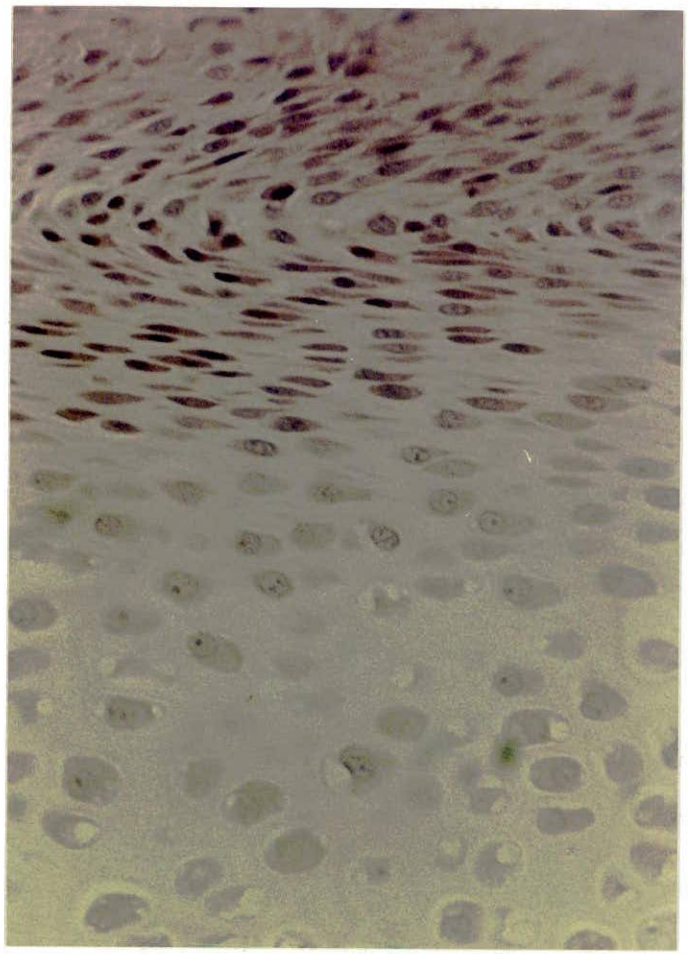


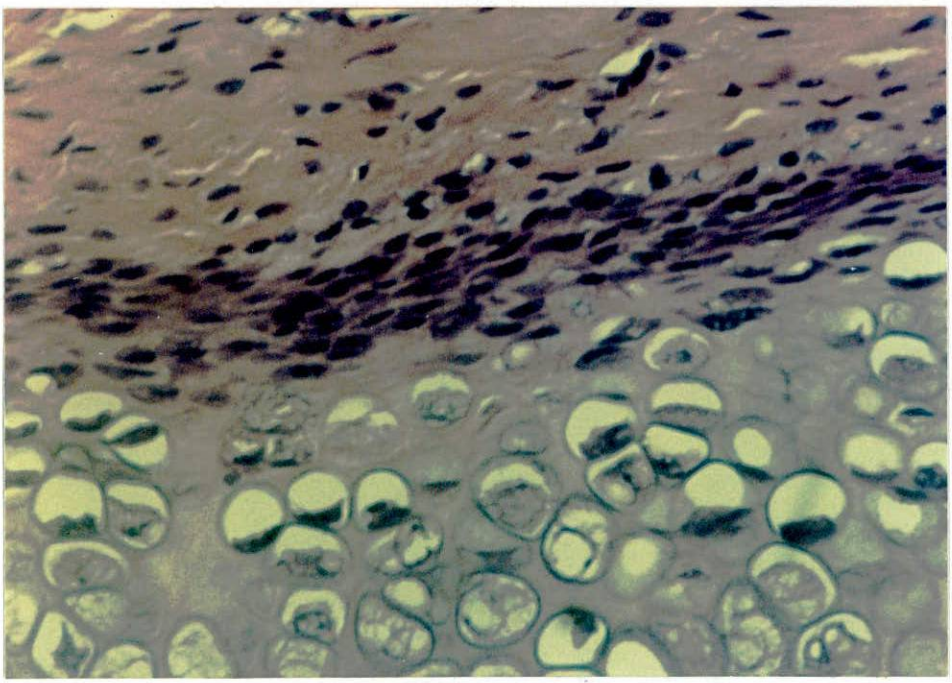
PHOTO IX

A 40x microphotograph of a section corresponding to the hyperpropulsion-only group. Note the good representation of the different layers and the increase in the dimensional features of the cartilage. Cells are loosely arranged.

PHOTO X

A 40x microphotograph of a specimen corresponding to the surgery-only group.Note the compact arrangement of hyperchromatic prechondroblasts in thegrowth zone. The functional zone has practically disappeared and the hypertrophic chondroblasts are well arranged.





C.-Hypertrophic Zone

The statistical analyses and mean values are provided in Tables IIIC-1 and IIIC-2 and mean values are represented in Figures IIIC-1 and IC-1.

No statistically significant difference among the mean values of the four groups was found for the hypertrophic layer in the second phase of the experiment. Paiwise comparisons were done using the Scheffe's procedure and the Tukey-HSD test, confirming that no two groups were different at a statistically acceptable level.

D.-Total Measurements

The statistical analyses and mean values are provided in Tables IIID-1 and IIID-2 and mean values are represented in Figures IIID-1 and ID-1.

As in the growth and functional chondroblast zone, the group III presented the greatest values, as it would be expected since this is only the addition of the previous values.

The other groups as ordered in relation to their numerical values follow the same order reported for the growth and functional layers. The surgical groups tend to hold the lowest values, as expressed for the layers previously analyzed.No statistically significant difference among the mean values of the four groups was found for the hypertrophic layer in the second phase of the experiment. Paiwise comparisons were done using the Scheffe's procedure and the Tukey-HSD test, confirming that no two groups were different at a statistically acceptable level.

PHASE II-ANALISIS OF CELLULARITY

Total cell counts of each of the layers of the condylar cartilage in the different groups at the end of the experimental period is as follows. A one-way ANOVA was carried out in order to evaluate differences in the means obtained from the different groups.

Variable ML2 MEASUREMENTS-FUNCTIONAL ZONE

By Variable GROUP

Analysis of Variance

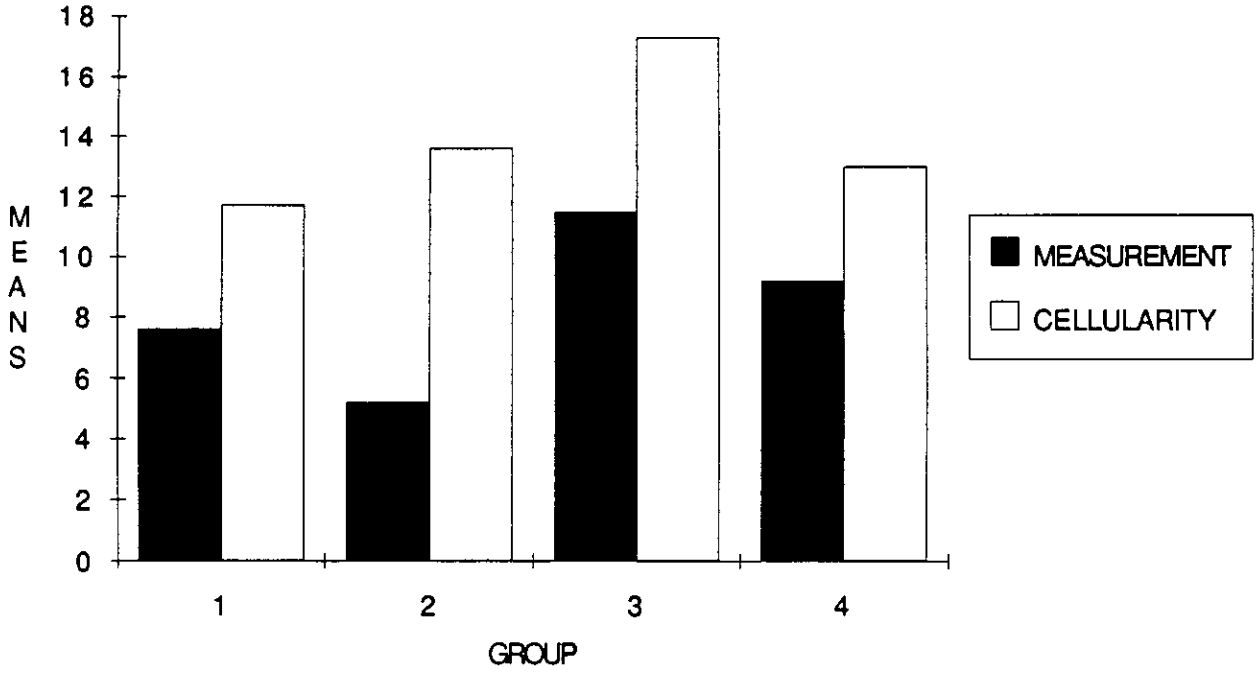
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	146.4046	48.8015	4.7592	.0100
Within Groups	23	235.8429	10.2540		
Total	26	382.2474			

Table IIIB-1. One-way Analysis of Variance of the mean measurements of the vertical height of the functional chondroblast zone. The analysis os the differences among the four groups is provided (p=0.0100).

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	7.6000	4.0846	1.6675	3.3135	To	11.8865
Grp 2	7	5.2857	2.3695	.8956	3.0943	To	7.4772
Grp 3	7	11.5571	2.3670	.8947	9.3680	To	13.7463
Grp 4	7	9.2429	3.7665	1.4236	5.7595	To	12.7262
Total	27	8.4519	3.8343	.7379	6.9351	To	9.9686

Table IIIB-2. Representation of the mean measurements of the vertical height of the functional chondroblast zone. The means calculated for the different groups are presented.

FIG. IIIB-1 FUNCTIONAL ZONE-MEASUREMENT VS CELLULARITY. PHASE II



Variable ML3 MEASUREMENTS-HYPERTRL ZONE

By Variable GROUP PHASE TWO

Analysis of Variance

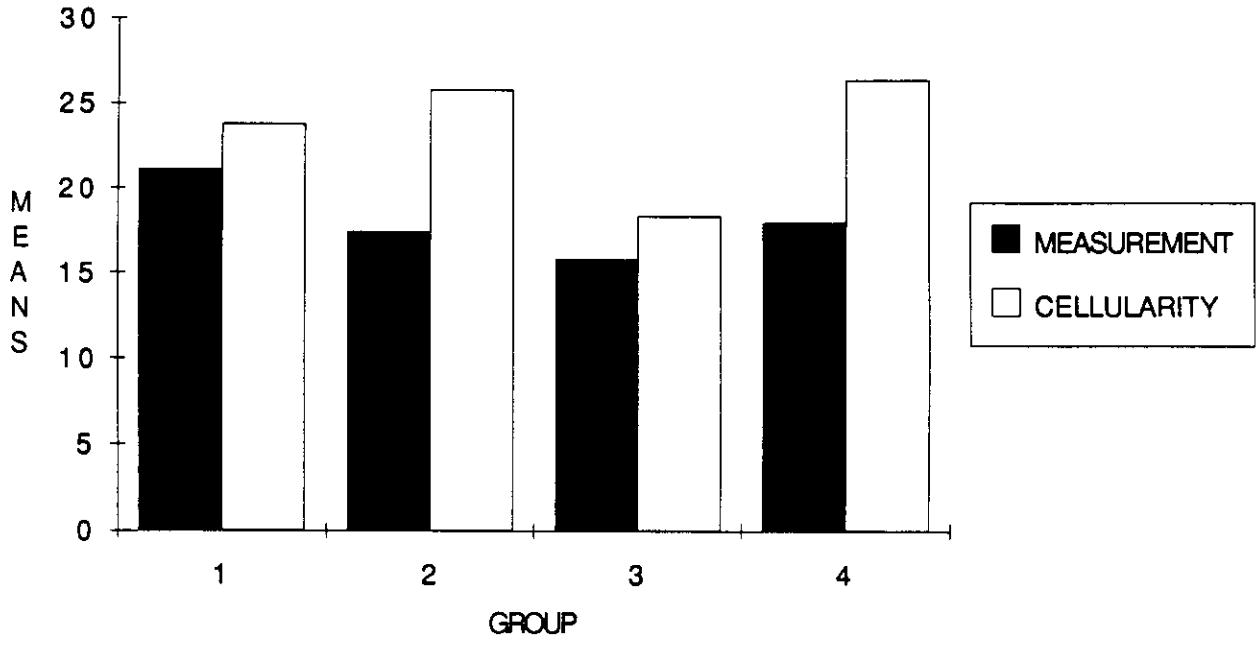
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	96.2212	32.0737	1.3220	.2914
Within Groups	23	558.0055	24.2611		
Total	26	654.2267			

Table IIIC-1. One-way Analysis of Variance for the mean measurements of the vertical height of the hypertrophic zone. The analysis of the differences among the four groups is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	21.1833	6.3250	2.5822	14.5458	To	27.8209
Grp 2	7	17.4714	5.6973	2.1534	12.2023	To	22.7405
Grp 3	7	15.8143	4.2530	1.6075	11.8809	To	19.7476
Grp 4	7	18.0714	3.0192	1.1412	15.2791	To	20.8637
Total	27	18.0222	5.0162	.9654	16.0379	To	20.0066

Table IIIC-2. Representation of the mean measurements of the vertical height of the hypertrophic zone. The means calculated for the different groups are presented.

FIG. IIIC-1 HYPERTROPHIC ZONE-MEASUREMENTS VS CELLULARITY.
PHASE II



Variable MTOTAL MEASUREMENTS-TOTAL

By Variable GROUP PHASE TWO

Analysis of Variance

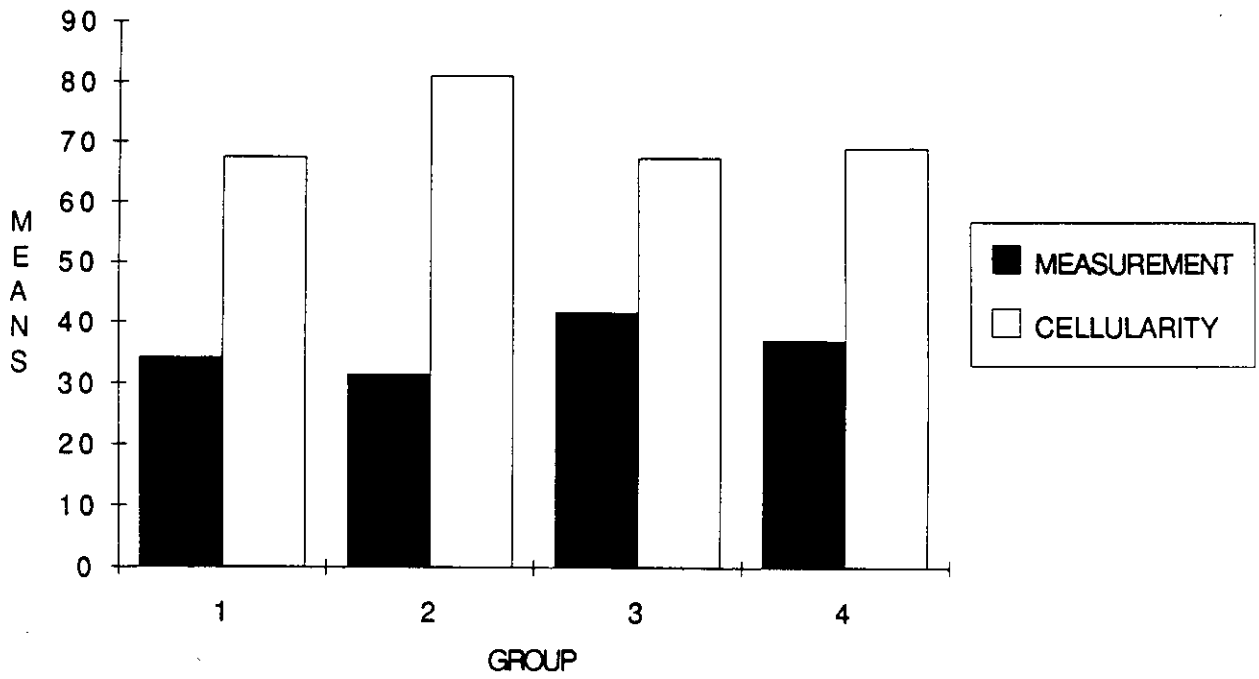
Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	401.2119	133.7373	2.2579	.1087
Within Groups	23	1362.2800	59.2296		
Total	26	1763.4919			

Table IIID-1. One-way Analysis of Variance for the mean measurements of the total vertical height of the cartilage. The analysis of the differences among the four groups is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	34.2000	9.4746	3.8680	24.2572	To	44.1428
Grp 2	7	31.5429	9.0065	3.4041	23.2133	To	39.8724
Grp 3	7	41.7714	6.0290	2.2788	36.1955	To	47.3473
Grp 4	7	37.2857	5.8970	2.2289	31.8319	To	42.7395
Total	27	36.2741	8.2357	1.5850	33.0161	To	39.5320

Table IIID-2. Representation of the mean measurements of the total vertical height of the cartilage. The means calculated for the different groups are presented.

FIG. IIID-1 TOTAL CARTILAGE-MEASUREMENT VS CELLULARITY. PHASE II



A.-Growth Zone

The statistical analyses and mean values are provided in Tables IVA-1 and IVA-2 and mean values are represented in Figures IIIA-1 and IIA-1.

The surgery-only group (II) showed the highest cell counts for the proliferative layer; the combined treatment group exhibited somewhat lower values, followed by the hyperpropulsor-only group; the control group showed the lowest cell counts.

These differences between the groups were statistically significant (p=0.001). Pairwise multiple comparisons revealed very significant values for the differences between the surgical group (II) and each of the other three groups. The same level of significance was not found for comparisons between the remainig three groups (group II excluded), which presented relatively close values.

A global vision of the order presented by the four groups in relation with the values obtained in the measurements carried out on this layer, permits to conclude that there is an inversion from what was observed at the end of the first phase of the experiment. The greatest-to-least sequence sen in this parameter in this phase i.e. II, I, III, IV is the opossite of what is observed during the first phase. The implications and significance of these changes will be properly analyzed later.

B.-Functional Chondroblast Zone

The statistical analyses and mean values are provided in Tables IVB-1and IVB-2 and mean values are represented in Figures IIIB-1 and IIB-1.

No statistically significant difference among the mean values of the four groups was found for the functional chondroblast layer in the second phase of the experiment. Paiwise comparisons were done using the Scheffe's procedure and the Tukey-HSD test, confirming that no two groups were different at a statistically acceptable level.

C.-Hypertrophic Zone

Variable CL1 CELLULARITY-GROWTH ZONE

By Variable GROUP PHASE TWO

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	603.8341	201.2780	7.3806	.0012
Within Groups	23	627.2400	27.2713		
Total	26	1231.0741			

Table IVA-1. One-way Analysis of Variance of the mean values for the cell counts of the growth zone. The analysis of the differences among the four groups is provided. The tes is statistically significant (p=0.0012).

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean
Grp 1	6	32.0000	2.0736	.8466	29.8239 To 34.1761
Grp 2	7	41.6571	7.1510	2.7028	35.0436 To 48.2706
Grp 3	7	31.7143	4.5264	1.7108	27.5281 To 35.9005
Grp 4	7	29.6286	5.4159	2.0470	24.6197 To 34.6375
Total	27	33.8148	6.8811	1.3243	31.0928 To 36.5369

Table IVA-2. Representation of the mean values for the cell counts of the growth zone. The means calculated for the different groups are presented.

Variable CL2 CELLULARITY-FUNCTIONAL ZONE

By Variable GROUP PHASE TWO

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	118.6088	39.5363	2.3085	.1032
Within Groups	23	393.9112	17.1266		
Total	26	512.5200			

TABLE IVB-1. One-way Analysis of Variance for the mean values of the cell counts of the functional chondroblast zone. The analysis of the differences among the four groups is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	11.7167	4.4220	1.8053	7.0762	To	16.3572
Grp 2	7	13.6429	4.6439	1.7552	9.3480	To	17.9378
Grp 3	7	17.3857	4.3884	1.6587	13.3271	To	21.4443
Grp 4	7	13.0571	2.9211	1.1041	10.3556	To	15.7587
Total	27	14.0333	4.4399	.8545	12.2770	To	15.7897

Table IVB-2. Representation of the mean values for the cell counts of the functional chondroblast zone. The means calculated fro the different groups are presented.

Variable CL3 CELLULARITY-HYPERTR. ZONE

By Variable GROUP PHASE TWO

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	281.3381	93.7794	2.3891	.0950
Within Groups	23	902.8226	39.2532		
Total	26	1184.1607			

Table IVC-1. One-way Analysis of Variance for the mean values of the cell counts of the hypertrophic zone. The analysis of the differences among the four groups is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	23.8167	5.1176	2.0892	18.4462	To	29.1872
Grp 2	7	25.8429	8.1047	3.0633	18.3473	To	33.3384
Grp 3	7	18.4000	5.4592	2.0634	13.3511	To	23.4489
Grp 4	7	26.4429	5.7581	2.1764	21.1175	To	31.7682
Total	27	23.6185	6.7487	1.2988	20.9488	To	26.2882

Table IVC-2. Representation of the mean values of the cell counts of the hypertrophic zone. The means calculated for the different groups are presented.

Variable CTOTAL CELULARITY-TOTAL

By Variable GROUP PHASE TWO

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	3	896.6352	298.8784	2.0810	.1306
Within Groups	23	3303.3648	143.6246		
Total	26	4200.0000			

Table IVD-1. One-way Analysis of Variance for the mean values obtained in the total cell counts. The analysis of the differences among the four groups is provided.

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean		
Grp 1	6	67.5333	6.8550	2.7985	60.3396	To	74.7271
Grp 2	7	81.1429	17.3897	6.5727	65.0601	To	97.2256
Grp 3	7	67.5000	11.2668	4.2584	57.0800	To	77.9200
Grp 4	7	69.1286	9.0586	3.4238	60.7508	To	77.5064
Total	27	71.4667	12.7098	2.4460	66.4388	To	76.4945

Table IVD-2. Representation of the mean values of the total cell counts. The means calculated for the different groups are presented.

The statistical analyses and mean values are provided in Tables IVC-1and IVC-2 and mean values are represented in Figures IIIC-1 and IIC-1.

The control group tends to present the largest values, followed by the surgical groups, that do not show very statistically significant difference. No statistically significant difference among the mean values of the four groups was found for the hypertrophic layer in the second phase of the experiment. Paiwise comparisons were done using the Scheffe's procedure and the Tukey-HSD test, confirming that no two groups were different at a statistically acceptable level.

D.-Total Cellularity Counts

The statistical analyses and mean values are provided in Tables IVD-1and IVD-2 and mean values are represented in Figures IIID-1 and IID-1.

The overall cellularity counts showed largest values for group II; the means corresponding to the remaining three groups exhibited very close values in the overall analysis of cellularity. The pairwise multiple comparisons proved to be significant for the differences between the surgical group and any other of the remaing three. However, once the group II was excluded, comparisons established between the other groups did not have statistically significant differences.

INTERACTIONS BETWEEN PROCEDURES AND PHASES

The rankings of the different groups oobserved in the first phase of the experimental period differ from the ones that were found in the second phase, at the end of the experiment, both for the dimensional analysis as well as for the cellularity counts.

In order to identify possible correlations between findings in the first and second phases and the interaction with the different procedures represented by the different groups, a two-way ANOVA was carried out for each layer, both for the dimensional and cellularity

analyses. The results of the evaluation of these interactions and levels of significance are forwardly presented.

DIMENSIONAL ANALYSIS

A.-Growth Zone

The two-way ANOVA interactions carried out for PHASE and GROUP provided a very high level of statistical significance for the growth zone($p<0.0001$) (Table VA-1;Fig. IA-1).

B.-Functional Chondroblast Zone

The same level of statistical significance was found for the analysis carried out upon the observations in the functional chondroblast zone. (Table VB-1; Fig. IB-1)

C.-Hypertrophic Zone

In the hypertrophic zone, the independent consideration of the variable Group or procedure, as it was shown in the one-way ANOVA, was not significant. The independent consideration of the effect of the PHASE upon dimensional values was considered significant ($p=0.003<0.005$). The interaction of both variables in the two-way ANOVA did not offer significant results from the statistical point of view. This can be interpreted as the different procedures did not have a different effect in the groups in the different phases (Table VC-1; Fig. IC-1).

D.-Total Measurements

When considering total measurements, the significance in the interaction of PHASE and GROUP was very high, informing of the combined effect that both variables exerted upon the dimensional features of the cartilage (Table VD-1; Fig. ID-1)

ANALYSIS OF CELLULARITY

A.-Growth

The result of the interaction between GROUP and PHASE upon the total count of cells in the growth zone was highly significant statistically ($p<0.0001$). This analysis does not

ML1 MEASUREMENTS-GROWTH ZONE
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	165.310	4	41.327	9.288	.000
PHASE	.420	1	.420	.094	.761
GROUP	165.286	3	55.095	12.382	.000
2-way Interactions	143.191	3	47.730	10.727	.000
PHASE GROUP	143.191	3	47.730	10.727	.000
Explained	308.501	7	44.072	9.905	.000
Residual	133.490	30	4.450		
Total	441.991	37	11.946		

Table VA-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean measurements of the vertical height of the growth zone. The statistical significance (Significance of F) for the two way interactions is provided ($p<0.0001$)

ML2 MEASUREMENTS-FUNCTIONAL ZONE
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	192.894	4	48.223	5.833	.001
PHASE	156.110	1	156.110	18.882	.000
GROUP	36.983	3	12.328	1.491	.237
2-way Interactions	203.306	3	67.769	8.197	.000
PHASE GROUP	203.306	3	67.769	8.197	.000
Explained	396.199	7	56.600	6.846	.000
Residual	248.035	30	8.268		
Total	644.234	37	17.412		

Table VB-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean measurements of the vertical height of the functional zone. The statistical significance (Significance of F) for the two way interactions is provided ($p<0.0001$)

ML3 MEASUREMENTS-HYPERTR. ZONE
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	269.760	4	67.440	3.103	.030
PHASE	206.670	1	206.670	9.509	.004
GROUP	71.830	3	23.943	1.102	.364
2-way Interactions	28.340	3	9.447	.435	.730
PHASE GROUP	28.340	3	9.447	.435	.730
Explained	298.099	7	42.586	1.959	.095
Residual	652.044	30	21.735		
Total	950.143	37	25.680		

Table VC-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean measurements of the vertical height of the hypertrophic zone. The statistical significance (Significance of F) for the two way interactions is provided (p=0.730).

MTOTAL MEASUREMENTS-TOTAL
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	781.946	4	195.486	3.974	.011
PHASE	687.611	1	687.611	13.979	.001
GROUP	84.846	3	28.282	.575	.636
2-way Interactions	541.303	3	180.434	3.668	.023
PHASE GROUP	541.303	3	180.434	3.668	.023
Explained	1323.249	7	189.036	3.843	.004
Residual	1475.638	30	49.188		
Total	2798.888	37	75.646		

Table V D-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean measurements of the total vertical height of the cartilage. The statistical significance (Significance of F) for the two way interactions is provided (p=0.023)

CL1 CELLULARITY-GROWTH ZONE
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	364.276	4	91.069	3.872	.012
PHASE	333.331	1	333.331	14.170	.001
GROUP	38.083	3	12.694	.540	.659
2-way Interactions	1520.612	3	506.871	21.548	.000
PHASE GROUP	1520.612	3	506.871	21.548	.000
Explained	1884.887	7	269.270	11.447	.000
Residual	705.687	30	23.523		
Total	2590.574	37	70.016		

Table VI A-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean values of the cell counts of the of the growth zone. The statistical significance (Significance of F) for the two way interactions is provided ($p=0<0.0001$)

CL2 CELLULARITY-FUNCTIONAL ZONE
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	126.751	4	31.688	2.322	.080
PHASE	45.241	1	45.241	3.315	.079
GROUP	78.845	3	26.282	1.926	.147
2-way Interactions	131.660	3	43.887	3.215	.037
PHASE GROUP	131.660	3	43.887	3.215	.037
Explained	258.410	7	36.916	2.705	.027
Residual	409.465	30	13.649		
Total	667.875	37	18.051		

Table VI B-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean values of the cell counts of the of the functional zone. The statistical significance (Significance of F) for the two way interactions is provided ($p=0.037$)

CL3 CELLULARITY-HYPERTR. ZONE
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	416.045	4	104.011	3.266	.025
PHASE	118.716	1	118.716	3.727	.063
GROUP	293.797	3	97.932	3.075	.043
2-way Interactions	77.361	3	25.787	.810	.499
PHASE GROUP	77.361	3	25.787	.810	.499
Explained	493.406	7	70.487	2.213	.061
Residual	955.488	30	31.850		
Total	1448.894	37	39.159		

Table VI C-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean values of the cell counts of the of the hypertrophic zone. The statistical significance (Significance of F) for the two way interactions is provided (p=0.499).

CTOTAL CELULARITY-TOTAL
BY PHASE
GROUP TWO-WAY ANOVA

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	721.899	4	180.475	1.598	.200
PHASE	502.967	1	502.967	4.455	.043
GROUP	229.239	3	76.413	.677	.573
2-way Interactions	2809.826	3	936.609	8.295	.000
PHASE GROUP	2809.826	3	936.609	8.295	.000
Explained	3531.726	7	504.532	4.468	.002
Residual	3387.336	30	112.911		
Total	6919.062	37	187.002		

Table VI D-1. A two-way ANOVA for the interactions between the main effect PHASE and the main effect GROUP (experimental procedure) was conducted for the mean values of the total cell counts of the of the cartilage. The statistical significance (Significance of F) for the two way interactions is provided (p<0.0001)

offer any doubt of the combined effect of interaction of both features (or variables) upon the cellular activity in the growth zone (Table VI A-1; Fig. IIA-1).

B.-Functional Chondroblast Zone

The two-way Interactions conducted here were found to be statistically significant, informing that both GROUP and PHASE jointly influence the cellularity of this layer (Table VI B-1; Fig. IIB-1)

C.-Hypertrophic Chondroblast Zone

As to the Hypertrophic Chondroblast Zone, the individual effects of PHASE or GROUP was considered significant but the interactions did not show the same level of statistical acceptability.(Table VI C-1; Fig. IIC-1)

D.-Total Cellularity

The level of significance for the interactions between the two factors, GROUP and PHASE , as affecting the total counts was extremely high. The result of the test confirmed that the combined effect of the experimental procedures performed in our study certainly interact with the time factor represented by the variable PHASE of the experiment, affecting in a differnt fashion the animals depending on the period of the experiment. (Table VI D-1; Fig. IID-1).

DISCUSSION

Numerous investigations presenting various degrees of evidence relative to the increment of growth that can be obtained in the condylar cartilage and condylar process in animals undergoing experimental mandibular protrusion have been reported over the past two decades. As shown earlier in this report, these investigarions have been subject to controversy in the forum of craniofacial growth and development. Moreover, while supporting opinions have been recently been expressed (McNamara, 1978, 1979; Owen, 1989; Petrovic, 1990), intense controversy seems to surround the notions about the alterability of mandibular growth through the use of hyperpropulsion appliances. Clinical trials carried out in humans as well as experiments conducted with various species of mammals seem to support the notion that amount and direction of the growth of the condylar process and mandibular ramus can be controlled to some extent. Even though there seems to be some agreement regarding the alterability of craniofacial growth through the use of functional appliances in humans or, analogously, with instruments or devices inducing experimental mandibular protrusion in animals, the intimate mechanisms involved in the local control of growth continue to be the focus of investigation and debate. The present investigation was conducted in order to evaluate the role played by the periosteum in regulating growth of the mandible and its condylar process.

The experimental procedures used in this study in order to achieve the purpose just described can be summarized as follows. A bilateral surgical periostomy around the neck of the condylar process was conducted in normal growing 21-day-old rats and a hyperpropulsion device was bonded onto the upper incisors in order to achieve an anterior repositioning of the mandible of 2 mm of sagittal advancement and a bite opening of 1 mm., in the group of combined treatment (group I). The two procedures (surgery and appliance)

were combined in order to determine whether the experimental manipulation of the periosteal envelope surrounding the mandibular condylar process modifies in a significant manner growth in animals undergoing experimental mandibular hyperpropulsion. In the second group (group II) only the surgical procedure was performed. In the next group (III) only the hyperpropulsion device was utilized. The effect of the device on growth of the mandible and its condylar cartilage was evaluated. A fourth group (Group IV) in which only a bilateral sham surgery was performed, served as control.

In each animal, a quantitative evaluation of the dimensional features of the layers forming thecondylar cartilage was performed. Individual measurements of the linear height i.e. thickness as well as an area analysis and cell counts were carried out for every layer as reported in the Materials and Methods section. The results obtained through use of these mnethods is summarized below. At the end of the first phase of the experimental period (first two weeks), comparisons between the values obtained in the dimensional analysis of the growth zone and hypertrophic zone for the different groups did not show statistically significant differences (Tables IA-1, IA-2, IC-1, IC-2). The dimensional values obtained for the functional chondroblast zone and for the measurement of the total height of the cartilage, indicated greatest figures for the surgical groups and lesser for group III, the control presenting values in between the previous ones (Tables IB-1, IB-2, ID-1, ID-2).

The analysis of cellularity at the end of the first phase of the experimental period indicated that the control group had the greatest cell counts in the growth zone and functional chondroblast zone. Differences for the mean values of cell counts in the hypertrophic zone of the groups were not statistically significant. The surgery-only group presented the lowest values and the two groups undergoing mandibular hyperpropulsion (groups I and III) exhibit values between the control group and the surgery-only group (group II) (Tables II from A to D; Figs II from A to D). The total cellularity analysis (including all layers in

the counting) presented similar data, indicating a very high level of significance in the differences between the mean values of the control group and any other one, specially the surgery-only group.

At the end of the second and final phase of the experiment (five weeks), the dimensional values for the growth zone and the functional chondroblast zone provided a similar order of the groups: the greater values corresponded to group III, followed by the control group (IV) and the surgical groups (I and II) presented the lowest values. The total measurements of dimensions presented similar values. The differences for the hypertrophic zone were not significant.

The cell counts at the end of the second phase indicated that all experimental groups had greatest values than controls in the growth zone; the surgical groups exhibited higher figures than group III. In the functional chondroblast zone, the differences were not significant, as they were not significant for the hypertrophic zone either. The total cellularity counts exhibited similar values to the ones presented for the growth zone (Tables IV D-1 and IV D-2).

Similarly, a two-way analysis of variance was carried out in order to determine the interaction of the factor PHASE and the factor GROUP (experimental procedure) for each layer in both the thickness measurements and the cell counts.

In the following evaluation of our results, it will be argued that integrity of the periosteum is necessary as a mechanism of local control and that the periosteum acts as an indirect local controller of the process of growth on the condylar cartilage. It also seems that experimental manipulation of the integrity of the periosteal membrane of the condylar process as well as alteration of the physical, biological and environmental conditions of the periosteum can affect growth of the cartilage of the mandibular condyle.

In addition to that, there is a considerable body of evidence indicating that the periosteum is common source of the cellular populations of both the cartilage of the mandibular condyle and the osteogenic population of cell of the periosteal envelope involved in the mechanisms of aposition and resorption. In this perspective, the nature of the control mechanism by which the periosteum influences growth of the condylar cartilage could be mechanical (Biophysical), citohistological (histodifferentiation of germinal cells called skeletoblasts) or both. Consequently, rupture of the integrity of the periosteal membrane or the changes of the conditions of the periosteal envelope, can affect the growth activity of the mandible through a double pathway by:

1-Introducing changes in the *status quo* of the biophysical and biomechanical conditions of the periosteum

2-Affecting the proliferative capability of the periosteum, reducing its capacity to nourish the cellular populations of both the condylar cartilage as well as the osteogenic layer of the bone surface of the mandible and by influencing the capacity of histodifferentiation of the germinal cells contained in the marginal zone of the periosteum, close to the articular surface of the condylar cartilage.

The results of our study allow us to speculate that alterations of the mechanical environment or modification of circumstances affecting the biophysical conditions of the periosteum do seem to produce a modification in the growth rate of the condylar cartilage. This notion is supported by of the significant differences we have observed for the growth parameters between experimental and control groups.

Because both surgical groups presented the lowest cellularity levels at the end of the first experimental period (Tables II from A to D; Figs II from A to D), it can be inferred that interruption of the continuity of the periosteum and subsequent stripping produces a

decrease in cellularity in the condylar cartilage, as expressed by the fact that both . Although, the dimensional features, like the vertical height of the growth zone does not seem to be affected in the same way, since the differences among groups for this parameter was not found to be significant (Tables I from A to D; Figs. II from A to D). Upon heling of the periosteum an important increase of the growth rate is detected. The cellularity level in the surgical groups (I, II) is significantly greater than in the other two groups for the growth zone and for the total counts (Tables IV from A to D). From these observations it can be speculated that a modification (decrease) in the tension generated upon the periosteum by muscular and capsular attachments (soft tissue structures with attachment upon the periosteal surface) by means of surgical periostomy resulted in a severe drop (or significant decrease) in the growth activity as expressed in terms of cell counts in the growth zone of the condylar cartilage.

Under our working hypothesis, a surgical periostomy would reduce the tension or compression exerted by the periosteal membrane. Likewise, it would alter the effect transmitted by the soft tissue attachments on the periosteal surface. Therefore, if in fact the increase in the tensional gradient of soft tissue structures results in a diminution of the compressive inhibitory effect of the periosteal tension, the surgical resection of the periosteum would interrupt the transmission of this effect to the periosteal surface. When the tension of the periosteum presumably regains normal values after the healing process is completed, the cellular activity noticeably increases in the growth zone as to exceed the values observed in non-surgical groups (TablesIV from A to D)

It is possible to speculate in light of our findings that the surgical manipulation of the periosteum induces a decrease in the level of tension of the periosteal membrane. This notion seems consistent with the finding that both surgical groups exhibit comparable values in the cellularity index during the first experimental period (first two weeks), even though one of the surgical groups (group I) was undergoing mandibular protrusion. The most depressed

cellularity values correspond to group II (surgery only) ,whereas the use of the hyperpropulsor in group I seems to introduce a difference in the cellularity levels present in the condylar cartilage, as indicates the greater values in group I at the end of the first phase. With the exception of the hypertrophic zone, where the values for the different groups were not statistically different, the pairwise comparisons revealed that the differences of the mean values obtained for the cellularity level in all layers, as well as the total counts, were statistically significant at the end of the first phase. The dimensional analysis did not reveal statistically significant differences at the end of the first phase. The difference in the cellularity levels seem to indicate that the different experimental procedures affected in fact the growth activity during that phase. It is tempting to hold the use of the appliance responsible for the differences found in the cellularity level between the two surgical groups (I, II) (Tables II from A to D; Figs. II from A to D).

Upon completion of the healing process of the periosteum, group II (surgery only) seems to experience a greater increment in the cellularity than group I (surgery and hyperpropulsion). In both surgical groups, the increment in cellularity is clearly noticeable and greater than in the control group (group IV). The analysis of the differences for the mean values of cellularity obtained in the growth zone indicated that the values obtained for group II were significantly greater than values for group I, a very high level of statistical significance (Table IIA-1 and IIA-2). The dimensional analysis also shows significantly greater values, from the statistical point of view, for group II than for group I in the growth zone and total measurements. It is conceivable to conclude that appliance use produces a transitory and relative slow down of the growth rate in the group of combined tratment (group I). During the period of disrupted periosteum, no statistically significant differences were observed in the two surgical groups (I and II) relative to the dimensional analysisand only a low level of significance was found regarding the cellularity. This lack of statistically significant difference in

the values of the two surgical groups during the first experimental period lead us to conclude that the appliance wearing factor (or, that the use of the appliance) does not introduce a significant difference in the growth activity ,as estimated by our parameters, when the periosteum has been resected and stripped or when the integrity of the periosteum has been altered. Conversely, when the restoration of the periosteum has taken place *ad integrum* , presence of the hyperpropulsor seems to have a transitory effect of decreasing the growth activity, as expressed by the significantly lower values of the group I (surgery-hyperpropulsion) relative to the group II (surgery only). The difference is observed for both the dimensional and the cellularity analysis, specially in the growth zone (Tables III and IV from A to D).

A transitory decrease of the values of the growth parameters was also observed in the group of animals undergoing hyperpropulsion only (group III) and has been repeatedly documented in the literature (Charlier, 1972; Petrovic and Stutzman, 1973, 1976, 1985, 1990; De Groote, 1985). Indeed, following our comparative analysis of the results observed in the different groups, it can be concluded that the use of the hyperpropulsion appliance on a full-time basis induces a transitory fall in the growth activity. This effect is expressed by statistically significant lower values of the dimensional features of the condylar cartilage in group III relative to controls in the first phase of the experiment. Likewise, the cellularity counts at the end of the first phase of the experiment were lower for group III than for the control group (IV), although the statistical significance of the difference for the mean values varied in the different layers and was not significantly high in all layers (Tables II from A to D). This transitory decrease of the growth parameters is followed by an increase of their values from the second to the fifth week. In fact, (Tables III from A to D), group III exhibited values significantly higher than any other group for the dimensional analysis at the end of the experiment. The cellularity counts followed the same trend and exhibited greater values for

group III than for controls in all layers, although the difference was not always statistically significant (Tables IV from A to D). Thus, it can be concluded from our results that mandibular hyperpropulsion performed by means of appliances which allow preservation of a physiologic range of movements of the mandible can induce an increase in growth of the mandibular condyle. Considering the nature of our data as well as reasons articulated in the Methods section, it appears that our appliance worked as expected and that our results can be compared to the ones reported by different authors.

The group undergoing surgery and hyperpropulsion exhibited a trend that can be summarized as follows: During the immediate postsurgical period, a release in tension that presumably takes place in the periosteum, produces a decrease of cellularity in the condylar cartilage. This trend is somewhat similar to that seen in group II. Upon healing of the periosteum, a significant increase in the cellular presence can be observed in group I. This increase, especially in the growth zone, is probably reinforced by the appliance use (Tables IV from A to D). The statistical significance for the differences between group I and group IV (controls) is not as high as the significance for the differences between group II and group IV, as it has been previously mentioned. Therefore, it seems that the hyperpropulsor appliance does not bring about any further growth in those animals which underwent surgical periostomy. The appliance does not contribute to a significant further increase of growth relative to the increment related to the healing. It can be argued that the increment in cellularity related to the healing and expressed as a rebound effect is of greater magnitude than the growth stimulation effect related to the use of the appliance. An alternative to this explanation is that the effect of the appliance is being reversed from a transitory decrease observed at the beginning of the experiment (end of first phase) to a growth stimulation effect. Therefore, the increments related to the use of the device might have not reached their fully expression and remained relatively masked by the posthealing growth stimulation.

As a consequence of surgical intervention, tension thought to be normally generated by the intact periosteum would be modified. Moreover, tension assumed to be transmitted by the menisco-capsular attachments as a consequence of mandibular protrusion would also be changed. Thus, our surgical manipulation prevented the periosteum from exerting its role of controller of growth activity. When histological and physiological integrity was presumebly restablished after the healing process was completed, an increased tension of the periosteum generated by the protrusive position of the appliance and transmitted by all menisco-capsular and muscle attachments, resulted in an increased of growth activity of the condylar cartilage which exceed those exhibited by controls and by the appliance-only animals . As to the latter, it can be speculated that the rebound effect in the growth activity after the healing process was completed is responsible for the difference in growth activity between these animals and the appliance -only group. Likewise, the histological contribution of the periosteum to the process of mitotic proliferation of the condylar cartilage was very possibly affected after the surgical section of the periosteum. The rebound effect in cellularity levels observed after the regeneration of the periosteal membrane introduces a difference between the growth activity observable in both groups of hyperpropulsion.

An alternative explanation for the difference between group I (surgery-hyperpropulsion) and group III could be attributed to differences in responses of the intact perioseum and the jus-healed periosteum to the appliance. Here, it is possible to argue in a double pathway, provided that we assume a double function of the periosteum in the condylar process: First, a function as an element involved in the biomechanical integration and transmission of growth messages; second, a function of histodifferentiation and contribution (incorporation of cells) to the cellular population of the proliferative layer of the condylar cartilage. Since this double function is supposed for the periosteal envelope, the surgical manipulation could have affected either one of them or, most likely, both. Likewise,

during the second phase of the experiment, the recently healed periosteum in group I could be responsive to the biomechanical stimulation carried out by the use of the appliance in a different manner than the intact periosteum of group III.

During the second phase (Tables IV from A to D; Figs II from A to D) the surgery-appliance group (I) presented cellularity values above group III and controls (group IV) but significantly below group II, statistically. In other words, the increase in cellularity levels experienced by animals with surgical periostomy after healing was significantly greater in those undergoing surgery alone (II) than in those combining surgery and hyperpropulsion (I). From this, we can infer that the healing of the periosteal membrane resulted in an increase of the cellularity level of the condylar cartilage. Moreover, it can be speculated that a rebound effect after healing was minimized in the group undergoing surgery and hyperpropulsion (group I) while it was fully expressed in the surgery-only group (II) as manifested by cellularity levels significantly greater at the end of the experiment (TablesIV from A to D). Therefore, the appliance does not provide a significant increase of growth in group I relative to group II, and also seems to have an inhibitory effect upon the increment in the cellularity related to the healing. However, as it has been discussed, the hyperpropulsion group (III) shows cellularity values above controls and dimensional values significantly greater ,statistically, than any other group during the second phase, what indicates a stimulatory growth effect of the appliance after the first phase of transitory inhibition has finished (Tables III and IV; Figs II and III).

From the information analyzed thus far, it is possible to conceive that the effect of biomechanical stimulation carried out by the functional appliances supposedly mediated by the increment of the tensinal level of the temporo-menisco-condylar apparatus and its action in the level of tension of the periosteal envelope, results in an increment of the cellularity values present in the proliferative layer of the condylar cartilage. Likewise, use of

hyperpropulsion alone produces an important increase of the dimensional features of the cartilage, and the difference with the rest of the groups was highly significant, statistically.

If we take the previous statements further, we can propose that the use of the hyperpropulsor device resulted in an increment of the growth activity of the condylar cartilage as expressed by the increase of observable cellularity in the growth zone of the cartilage. Nonetheless, in the combined-treatment group the effect of biological restoration of the periosteum introduces an increment of the growth values above the ones obtainable through the use of hyperpropulsion alone. This leads us to conclude that, in effect, the histological contribution of the periosteum at the level of the condylar process, in the perichondrial limit and grading onto the condylar neck, is of primordial importance in the proliferative activity of the mitotic *stratus* of the condylar cartilage.The relevance of such a contribution, understood as histodifferentiation and incorporation of cells with mitotic potential to the proliferative layer of the cartilage, seems clearly characterized by two basic phenomena identified in the analysis of our results . First, the abrupt drop of the cellular activity (Tables II; Figs I and II) after the surgical section that, supposedly, would have diminished considerably the potential contribution of the periosteum to the condylar cartilage. Second, the rebound effect observed in the cellularity analysis in both surgical groups resulted quantitatively more important than the increment obtained as a consequence of the treatment with hyperpropulsion appliances(Tables III and IV; Figs.II and III).

From what we have shown so far, there seems to be considerable evidence hinting that integrity of the periosteum develops a multiple and relevant role in the growth process of the mandible and its condylar cartilage. More specifically, it can be argued that the histological contribution of the periosteum might be of greater relevance than its role as an integrative element of the biomechanical stimuli and transformation of these stimuli in messages of stimulation of the growth process.

Functional activity has been proposed to be a necessary element in order to obtain growth stimulation through the use of hyperpropulsion appliances. In this view, our results allow us to conclude that our device permitted in effect a sufficient range of physiologic mobility. In the hypothetical case that the device had not permitted a certain range of physiologic mobility in our study, we can state that this factor did not prevent an increment of the growth activity of the animals undergoing hyperpropulsion.

This argument is equally valid when applied to the analysis of the differences between the group undergoing surgery alone and the group of combined procedures. In effect, the range of mobility present in group II is unavoidably greater than the range observed in group III (appliance only) since the appliance theoretically limited the range of mandibular mobility to a greater extent than the surgical procedure did. The relevance of this factor as being responsible of the possible differences between these two surgical groups becomes difficult to evaluate. Nevertheless, it seems conceivable that the possible limitations in the mandibular mobility secondary to the surgical manipulation could be one of the factors involved in the severe decrease of cellularity observed in the potsurgical phase. The mechanism for this diminution in the cellularity levels could be explained as the cumulative effect of two factors operating in the same sense. If tlimitations in mobility secondary to the surgical trauma are added to limitations caused by placement of the appliance, then it might be possible to understand the differences noted between the two surgical groups. It could be speculated that limitations in mandibular mobility introduce a difference in the final values of the surgery-only and the combined-treatment groups.

The control group shows the highest values of all the groups during the first stage of the experimental period, showing that experimental intervention, either surgical or the placement of the appliance, generates a decrease in the growth activity (Tables I and II; Figs I). When traumatic effects disappear or are minimized, both modalities of treatment result in

growth measures which exceed those seen in controls. It can be proposed, then, that the introduction of any experimental procedure alters, at least in a transitory way, growth observed in the different experimental groups.

The transitory decreases in cellularity observed following immediately placement of the hyperpropulsor in this study and documented in previous reports is poorly understood. We might agree with Petrovic's perspective stating that placement of a hyperpropulsor disturbs the mechanism of propioception sufficiently to block the adequate transmissionof growth stimulation messages. A period of transition is necessary in order to restore certain biological balance. Subsequently, stimuli generated by an appliance is adequately transmitted and integrated in the system of control and regulation of growth. From this moment on, eferent responses are generated, and unaltered morphfunctional balance is obtained. The analysis of our results is congruent with this interpretation, since the transitory decrease in cellularity seen in our results, is followed by a restablishment of growth activity, which continues to increase beyond the values seen in the control animals. On the other hand, our analysis of thickness of the proliferative layer of the cartilage in the group undergoing hyperpropulsion exclusively, reveals values below those presented by the controls, during the first phase of the experimental period. This order is inverted at the end of the experimental period (end of the 5th week), when the dimensions of vertical height of the growth zone are greater in the appliance-only group (Tables I and III; Figs I). The cellularity index as well as the thickness and surface analyses in the proliferative layer of the condylar cartilage (Tables IA , IIA, IIIA and IVA; Figs I and II), exhibit the same tendency during both the first and second phases of the experimental period, in the group undergoing hyperpropulsion alone.

The evaluation of the control group in relationship to the surgical group demonstrate that the cellularity holds in controls during the first phase of the experimental period, whereas

the surgical section originates an important decrease in the surgical groups. This order is exactly the opposite, after the period of healing of the periosteum, at the end of the second phase , when it is observed that the cellularity levels in surgical group considerably exceed those seen in controls (Table II and IV; Figs II and III). The dimensional analysis of the proliferative layer does not reflect values comparable to those obtained for the cellular counts. In fact, measurements of the thickness of the proliferative layer are greater for the surgical groups relative to controls in the first phase, and the differences are specially significant for group II (Tables IA; Figs IA). Again, this order is reversed at the end of the experimental period; thickness of the growth zone in the surgical groups is significantly less than in the control group, and the control group exhibit values lower than those measured in group III (appliance only) (Tables IIIA; Figs. II and III).

A global consideration of this data allow us to affirm that, in effect, both the modification of the cellularity and the dimensional changes in the mitotic layer, undergo a parallel and concordant evolution in the group of appliance only relative to controls, unlike the surgical groups, where the changes for one and other category, i.e. cellularity and thickness, seem to show directions which are clearly opposed.

A more detailed vision of these observations lead us to remark that, perhaps, the tendency of the changes in cellular activity and dimensional features of the condylar cartilage are similar, although both parameters do not change with a proper coordination in time. Indeed, it is possible to speculate in light of the Tables of these Results, that the tendency to diminish the growth activity manifested as a decrease in the cell counts of the proliferative zone, is noticeable immediately. From these results it can be inferred that the cellularity levels exhibit a quick response to the surgical manipulation, showing in the first phase a severe drop of the cellular levels of the proliferative zone. It is also possible to argue that the dimensional changes are more time-dependent and require a greater period of time to become evident. At

the end of the experimental period, the dimensional changes secondary to the surgical procedures become evident when, in contrast, the cellularity is showing already the rebound effect secondary to the periosteal regeneration. In other words, we can speculate than the surgical manipulation of the periosteum would carry out a decrease of both the dimensions and the cellularity of the cartilage. However, the decrease in the cellularity would be evident immediately, as noted by the lower values in cell counts of surgical groups at the end of the first phase (Tables II). In contrast, the dimensional features would undergo a slower change in such a way that the dimensional values would appear relatively unaffected at the end of the first phase and would only show decreases secondary to surgery at the end of the five weeks (Tables III; Figs I and II). At the end of the experiment, the cellularity of the surgical groups exhibits an increase supposedly corresponding with the healing of the periosteum. According to this interpretation, recovery of the dimensional values in surgical groups would have been evident in a longer period of observation. If, in effect, the tendency to the reestablishment of a greater degree of thickness in the mitotic compartiment could have been detected if we had increased the length of the experimental period or, whether such a tendency to the dimensional increment was not present, it is impossible to elucidate and only partial and speculative interpretations can be provided. However, it is conceiveble that this is the case and that would allow to explain the lack of coordination in time of the changes observed in one and other category, offering the misleading appearance of a lack of consistency in the behaviour of one and the other parameters.

As stated before, a noticeable consistency in changes observed in phase one and phase two between the different parameters analyzed is indeed present in the group of hyperpropulsion and it is tempting to assume that this consistency could be related to the integrity of the periosteal membrane. When the perioteum is intact, changes in the thickness,

surface and cellularity presumably preceed the dimensional changes in the proliferative layer of the mandibular condyle.

The lack of consistency that we found between the surgical groups in dimensional changes and mitotic activity has some precedents in earlier literature in studies of hyperpropulsion which did not involve surgical procedures . For example, Gaumond (1975, as cited by De Groote, 1984) reported an increment of the cellular activity after 60 days of treatment with hyperpropulsor, at a point where decreases in the dimensional parameters registered in the mandibles of the experimental animals is more noticeable (the drop is more severe, or simply the dimensional features reach the lowest point). Similar results have been reported by De Groote (1984). The interpretation of a possible inversion of an inhibitory effect upon growth was formulated in this case, although is possible to explain the phenomena, as we argue, in terms of a lack of correspondence in time between the changes undergoing by dimensional features and cellular activity. In the study conducted by Gaumond (1975), dimensional parameters continue to decrease until the 60th day of treatment, while cellularity values were already increasing at this moment. Differences in methodology as well as in appliance design and prescription between this study and those of Gaumond and De Groote could possibly account for time-related differences in the growth activity. Still, it is conceivable that the tendency of our results is the same, yet the lentgth of our experimental period is shorter than in these other studies.

Considering dimensional changes exclusively, it is possible to conclude that our study of hyperpropulsion exhibits the same tendencies reported by Petrovic, Stutzman and others in similar investigations. Dimensional changes, both linear and area measures, reflect the same tendency to a transitory decrease and later acceleration in the hyperpropulsion-only group, with this group outgrowing the contols by the end of the experiment. Such changes were also observed in the functional chondroblast zone and in the hypertrophic zone as well

as in the measurements of the total height of the condylar cartilage and measures of the total area. In the second phase of the experimental period, the situation is the same for every one of the layers of the condylar cartilage in the hyperpropulsion group: their values exceed those detected for the control group (Tables I and III; Figs. I and II).

With regard to the surgical groups, during the first experimental period their cellularity values were lower than those seen in controls in all the layers and in the total counts, with the sole exception of the hypertrophic layer, where the differences were not significant. Conversely, their dimensional values exceed those seen in controls. Here again, differences in the hypertrophic layer were not found to be statistically significant (Tables I and II).

As to the second experimental period, values for the surgical groups in underlying layers generally followed a trend demonstrated in the growth zone (Tables IV; Figs II and III) and were lower than those seen in controls in the dimensional analysis. Differences in the cellularity counts were statistically significant in the growth zone and in the values of the total counts, but not so in the functional and hypertrophic layers.

Comparative analyses of differencesobserved in the growth zone relative to the rest of the layers of the cartilage show that the differences are more statistically significant in the proliferative layer than in the others. The same trend observed in the growth zone can usually be identified in the other layers, but the differences do not have the same level of significance. From this,it can be suggested that changes observed in the growth zone do not take place with the same magnitude in other layers or might need more time to be manifest. It is not surprising that the response of the proliferative zone is not only faster but also more evident than in any other layer. In other words, it is probable that the mitotic compartiment is more susceptible to undergo important modifications upon alteration of the control mechanisms of growth .

The underlying layers are, to a good extent, an indirect manifestation of the phenomena with influence in the cellular activity of the condylar cartilage and, therefore, respond secondarily to changes in the proliferative zone. The reasons why the dimensional drop is uniform for all the layers in the group of surgery alone are not clear . Things are even more confusing if we take into consideration the observation that the presence of the hyperpropulsor during the phase in which the periosteum has healed does not seem to induce any additional increments in cellularity in the growth zone when compared to group II. Moreover, as already discussed , presence of the hyperpropulsor in group III seems to induce a similar stimulation of cellularity and dimensional features of the cartilage in both phases of the experiment. Fluctuations in cellularity in the groups during different phases of the experiment seem to be specially pronounced in the mitotic compartment of the condylar cartilage. It is in this proliferative layer where the changes are more evident between the first and the second period of our investigation. That was to be expected under our perspective, since we believe that it is in the growth zone where the influence of the regulating factors that physiologically control the growth activity of the condylar cartilage is specially noticeable. Therefore, experimentally induced changes should have a direct impact in the dynamics of the proliferative layer.

The correlation between the cellularity and the dimensional features in the functional and hypertrophic layers prompts some additional comments. The dimensional values at the end of the first experimental period for the proliferative zone were only significantly different for group II, as was presented in Tables I and Figs I and II . In the functional chondroblast zone as well as in the values for the total measurements, the order is similar and the differences significant for all the pairwise comparisons of all groups (Tables IB and ID). The same phenomena can be observed with the cellular counts; when the different groups are ranked according to the values obtained , the order is very similar for all the layers, including the total

counts (Tables II; Figs I and II). Even though the order of the groups is not the same for the two categories, i.e. dimensional measurements and cellularity counts, the differences in the order are practically constant for all layers of the condylar cartilage (Figs II and III). The notion has already been proposed that the tendency could be similar in both cases but that differences in time-related changes could occur. Such a misimpression underrepresenting correlations between dimensional changes and cellularity levels coul be created. At the end of the second experimental period the rank of the different groups vary from one layer to the other in both the dimensions and cell counts. Therefore, no correlation can be established. In this instance, this real lack of correlation between the cellularity and the dimensional changes is perfectly noticeable in the values presented in Tables I to IV and Figs IA-2 to ID-2.

As explained in the Results section , two-way analyses of variance were conducted in order to determine the interactions among the different procedures (represented by the different groups) and phases for every one of the growth parameters evaluated in this study.

As to the dimensional analyses, it was statistically observed that highly significant interaction occur between the factor Group and the factor Phase, in the evaluation of the figures for the growth zone and for the functional chondroblast zone (Tables V from A to D;Figs IA-2 to ID-2 and IIIA-1 to IID-1). This means that different procedures and phases jointly affect the dimensional features of the cartilage in the growth zone and functional chondroblast zone. Accordingly, procedures performed in the groups affected characteristics of the growth and functional zones differently in the two phases of the study. The same level of statistical significance can be found for the total measurements, whereas the hypertrophic zone did not show any significant values in the interactions (Tables VC and VD).

Analyses of Variance of the cell counts and the linear measures yielded similar results; i.e. cell counts were also jointly affected by procedures and phases. Alternatively

stated, cell counts in the different phases reflected different responses to the various procedures (Tables VI from A to D). These results are consistent with the fact that the biological conditions in different phases were also different. During the first phase, the periosteum was disrupted and undergoing healing process in the surgical groups. In group III, the first experimental phase corresponds with the period of adaptation and temporary decrease of the growth activity that is known to follow the placement of a hyperpropulsion appliance. The second phase corresponds to the period of growth stimulation, after the adaptation of the animal to the appliance is achieved. Our results are supportive of the notion that different responses occur to different procedures in Phase I and Phase II as expressed by the values cited (Tables V and VI, from A to D).

Concordance of the information obtained in the analysis of interaction with the information obtained in the individual analysis carried out in phase I and phase II, as well as the high level of statistical significance found for the interactions lead us to believe that the biological premises assumed (or biological conditions supposed) for phase one and two may be valid (periosteum disrupted versus healed or adaptation phase to the appliance versus growth stimulation phase). Besides, the traumatic component involved in any experimental manipulation will probably affect the growth activity and might generate a decrease in the growth rate, especially during the first phase. When the traumatic effect disappears, the effect of the experimental procedure may be more clearly observed.

An overall interpretation of our results suggests that, in effect, both surgical periostomy and hyperpropulsion, as well as the combination of both procedures can introduce a significant change in the growth process of the condylar cartilage. As to the biomechanical role played by the periosteal membrane in the transmission and integration of growth messages, there seems to be evidence hinting towards this notion. In the sequence of biomechanical stimuli which may regulate growth activity in the condylar cartilage, one of

the important elements seems to be the capsular unit and its temporo-menisco-condylar elements. Tensions in the capsular unit and transmitted to the condylar cartilage through the periosteal attachements of these elements in the condylar process seems essential in the regulation of the growth activity in this region. These normally occurring, physiologic tensions and their related signals can be manipulated experimentally.

Now it seems reasonable and appropriate to formulate the question: What is the mechanism by which changes in the tensional level of the soft tissue structures with periosteal attachment upon the condylar surface generate changes in the growth process of the condylar cartilage?. It is in this formulation of the problem of growth control in the condylar region where the notion has been proposed that the tensional changes originated or reflected in the menisco-capsular apparatus are transmitted to the periosteum covering the condylar region and that tension in the muscular structures and in the temporo-capsulo-condylar elements are translated to the periosteal envelope of the condylar process of the mandible. Therefore, changes in tension in the soft tissue structures with periosteal attachment in the condylar process could produce alterations in tension gradients in the periosteal envelope of the mandibular condyle. If these changes are of sufficient magnitude and duration, alterations in growth may ensue.

The above presented experimental model has an interpretative value and allows us to offer an explanation of the mechanism by which placement of a hyperpropulsion device produces a change in the growth process of the cartilage. Such an explanation could be formulated as follows: Anterior repositioning of the mandible results in an increment of the tension exerted upon the soft tissue structures of the capsular unit; this increment in capsular tension produces a corresponding alteration in the tensional level of the periosteal membrane of the condylar surface. This biomechanically induced periosteal tension is then converted into a signal, possibly of biochemical nature, which evokes a response at a cellular level. This

histological response is expressed as a modification of the growth characteristics in the mandibular ramus and its condylar process, such as rate, amount and direction.

This experimental model proposes the periosteum as a local controller of the process of growth on the condylar cartilage upon physiologic and the experimental conditions used in the present study. Indeed, it presents the periosteum as a key connecting or vinculating regulatory factor between the biological environment of the masticatory apparatus and the growth messages being processed in the condylar cartilage where a response can be detected or expected. However, the interpretation provided by this experimental model cannot be universally applied in the context of the craniofacial complex to explain the mechanism of local control of growth. Likewise, even though it provides some plausible answers , it poses a few difficult questions as well. Some of them have been subject of study over the last decade.

In one of the very few studies of surgical periotomy conducted in the mandibular condyle, Parks (1979) formulates the working hypothesis that the tension exerted by the periosteal membrane upon the condylar surface in resting conditions, carries out a restrictive effect on the growth of the condylar cartilage. The conceptual basis for Park's hypothesis lays on the fact that the results obtained in previous studies of periostomy conducted in long bones (Harks,1970; Crilly, 1972; Duterloo,1976) indicate an increment in the growth activity of the epyphiseal growth plate. Neither the project conducted by Parks (1979) nor the series of investigations carried out by Koski and Ronning in the course of the last decade have succeeded to demonstrate a similar reaction after the surgical periostomy of the condylar region. Differences in technique as well as in the length of the experimental period can account partially for that. In any case, is seems reasonable to speculate from the studies carried out in long bones and in the mandible that surgical manipulation of the periosteum induce changes in growth activity. Even though the specific characteristics of the changes

obtained in one case or the other differ, results of these studies seem to indicate that the periosteum has an effect of local integration of growth regulatory factors , both in the epiphyseal growth plate of the long bone and in the cartilage of the mandibular condyle.

Thus, it is tenable that the tension of the periosteum operates as a built-in component in a system of local integration of growth messages, both in the long bone and in the mandibular condyle. The effect of muscle function with periosteal attachment will exert its effect upon the growth plate or the condylar cartilage *via* the periosteum. Some other mechanical factors will also influence growth activity by conducting biophysical messages through changes in the periosteal tension. Such changes in periosteal tension will then be transformed into responses in the growth activity of long bones or the mandible. As to the mandible in particular, the contribution of the periosteum to the growth process of the condylar cartilage has been presented as being an increment in the mitotic activity of the proliferative layer as well as histodifferentiation and further incorporation of cells of periosteal origin to the proliferative layer of the condylar cartilage. In long bones, restrictive effects of tension of the periosteum has been proposed to explain experimental findings after sectioning of the periosteal envelope. The surgical release in tensions of the periosteum diminish the inhibitory effect, allowing additional growth of the growth plate.

In the mandible, surgical sectioning of the periosteal envelope of the condylar neck can affect the growth of the condylar cartilage in various ways. First, the contribution of the periosteum to the cellular population of the proliferative layer of the cartilage may have been disrupted. On the other hand, an induced decrease of tensional levels of the periosteum may exert an stimulatory or inhibitory effect, directly upon the cellular proliferation.

In effect, the collagenous belt that surrounds the articular surface of the condylar head has been proposed (Koski and Ronning, 1985) as an area of pressoreception. Under our perspective, such area of pressoreception could be capable of receiving and transmitting

biomechanical stimuli such as the level of tension of the periosteal membrane, as well as the index of mechanical stress of the articular surface. The latter , if properly characterized, could add valuable information to the control mechanism presumebly present in the articular unit, that tends to prevent the excessive mechanical loading of the articular surfaces. The mechanism has been hypothesized to work as a feed-back system detecting the excessive levels of mechanical stress within the joint and subsequently answering with readjustments in order to prevent the potential damage of the articular components. The pressoreception, if it is confirmed, would also facilitate some further understanding of the theory of underpressure of the articular capsule as an stimulating factor in the growth increase theoretically accomplished through the use of functional appliances in humans, or devices of mandibular hyperpropulsion in animal experimentation. Information about the mechanical conditions of the condylar surface could be integrated in the mechanism of growth control of the condyle and the mechanical stimuli could affect the proliferative activity of the cartilage not only through a direct action or effect on the articular side, but most importantly, through the effect upon the periosteal envelope.

In this scenario surgical interruption of the periosteum could affect the ability of the periosteum to nourish the cellular population of the condylar cartilage, and also influence the ability of the periosteum to integrate mechanical stimuli and transform them in a histological responses in the condylar cartilage or in the epiphyseal growth plate of the long bone. As to its role in the integration and transmission of mechanical stimuli, the role of the periosteum seems to have been only partially characterized both in the long bone and in the condylar cartilage.

In spite of the partial interpretations that have been previously reported in the literature , until we know more about the growth processes in the mandible and long bones, we can only speculate why there are differences between the two. Nonetheless, based on

our study it seems that the mandibular and long bone growth do resemble each other in some important ways. For example, the dimensional characteristics of the condylar cartilage during the phase of disrupted periosteum follow the same trend observed in the long bone.

One of the features which distinguishes the present work from other studies based on condylar surgical periostomies is our extension of the observation period to five weeks.of our study when compared to the ones that have preceeded in the limited series of surgical periostomies on the mandible, is that the period of observation is extended to five weeks. This permits an analysis of the changes observed in the different groups at the end of the second week, when the growth activity of the condylar cartilage is still presumebly influenced by the surgical procedure. But, importantly, this increase in the length of the experimental period has enabled us to undertake analyses of the changes in the different groups, once the regenerative process of the periosteum has been completed. By studying two-week and five-week groups it is possible to carry out a comparative analysis of the role of the periosteum under different biological and biomechanical conditions(disrupted periosteum in the first phase, and healed periosteum in the second phase). A better understanding of the intimate functioning mechanisms of the periosteal membrane in different experimental conditions, as well as the extension of the observation period should give us some insight into the apparent lack of consistency between the findings in the mandibular condyle and the ones in the epiphyseal growth plate of the long bones. Nonetheless, our awareness of notable differences in the biomechanical function, histological structure, developmental characteristics, patterns of growth and modality of maturation and ossification in the epiphyseal cartilage and the condylar cartilage prompt us to speculate cautiously: given these known differences, it is likely that there will be real differences in growth of these two structures.

Indeed, based on the outcome of the research conducted in condylar periostomies, it is conceiveable to state that the presence of a certain tensional level on the periosteal surface might, in fact, develop a role in the biomechanichal control upon the process of condylar growth without having necessarily a restrictive effect. In fact, the results of the most part of investigations involving the use of hyperpropulsion appliances seem to point in the opposite sense. Under the present hypothesis and under the model that has been presented above, tensional increments of the capsular unit and structures with periosteal attachment , would result in an increase in the periosteal tension that would induce growth in the condylar cartilage. From the biomechanical point of view, the only way to reconcile the meaning of these experimental findings would be to hypothesize that the nature of the force exerted in physiologic conditions by the periosteal envelope upon the condylar surface, is compressive rather than tensile. Therefore, the surgical incision would produce a release of inhibitory compressive forces. Conversely, use of a hyperpropulsion appliance would induce tensional traction conducted trough the soft tissue structures with periosteal attachment, what would result in a diminution of the inhibitory compressive effect of the periosteum in physiologic conditions. A decrease of the periosteal compression would occur as a consequence of the traction exerted by the soft tissue structures with periosteal attachment that would attempt to displace the periosteal envelope away from the condylar surface. The idea is seemingly consistent with the notion that tension induces aposition and compression promotes out resorption, even though the mechanism by which an increment in periosteal tension induces an stimulatory effect upon the condylar cartilage remains obscur or unexplained. This would be, supposedly, the connecting factor in the sequence of facts that we hypothesize, that should be subjected to further investigation. Finally, the present analysis can only attempt to establish a qualitative comparison. In fact, it is possible to articulate a discussion relative to the magnitudes and parameters present in both systems (condylar process and long bone) and

supposedly operative in the same line of function (or maybe better: supposedly involved in the same process), but we can hardly establish a quantitative comparison. The impossibility of quantifying certain magnitudes becomes ,at least, a limitation present in the study of our experimental models. Features such as the loading rate or the level of mechanical stress developed in the long bone or in the mandibular condyle exhibit differences, quantitatively substantial, that might induce, at least, a difference in the biological environment. Likewise, as reported by Parks (1979) the interstitial growth rate exhibited by long bones seems to be more rapid than the appositional mode of growth present in the cartilage of the mandibular condyle. The difference in the growth rate could be of re;levance in analyzing the changes that occurred in the condylar cartilage during a short period of time in comparison with the changes observed in the epiphyseal growth plate during a longer experimental period. Both factors, growth rate and length of the experimental period, seem to add up to make changes in the long bone more noticeable and easier to detect.

Another one of the unsolved problems in this experimental model are the oscilations in the levels of periosteal tension thought to occur during the physiologic mandibular excursions, and the trascendence of these changes in the local regulation of growth. If we accept the premise that the compressive forces are effectively present under physiologic conditions and have an inhibitory effect on the growth activity of the condylar cartilage, and that this inhibitory effect can be partially counteracted by by the placement of a hyperpropulsion device, it still remains to explain the relevance of the range of physiologic movement that allow the different appliances. Furthermore, it should be characterized the importance of preserving that range of functional movements of the mandible when the design of the appliance is carried out in order to accomplish an effective stimulation of the growth activity. Rees (1954, as cited by De Groote, 1984) first showed that during the physiologic movements of the mandible, condylar displacement within the

temporomandibular joint is followed by adaptive changes in other structures of the articular region in order to preserve a state of morphfunctional balance. Furthermore, measurements of the intracapsular pressure carried out in humans by Findlay (1964) demonstrated that upon opening an protrusion, variable amounts of negative pressure can be detected. In similar studies, Petrovic and Stutzman (1976, 1977) documented increments in tension of the posterior wall of the articular capsule and temporo-mandibular frenum during opening movements of the mandible and, quantitatively more relevant, during experimentally induced protrusion.

In light of these findings, adaptive changes secondary to the anterior repositioning of the mandible would tend to restablish the previous biomechanical conditions through local adjustments of anatomic and biomechanical type. It remains to be explained why these changes are not sufficient to minimize the effect of the propulsion appliance and to stimulate a growth of the condylar cartilage. Such growth would occur until a restablishment of the original biological balance has been obtained.

Another series of limitations in this study derived from technical-methodological aspects.The effective execution of the surgical procedure in the relatively small field of a Sprague-Dawley 21 day-old rat (the surgical openning was smaller than 10 mm) , posed some difficulties.

For example, it was impossible to surgically expose the medial side of the condylar region. This restriction limited wholesale stripping of the periosteum to the lateral side of the condylar process, which could be directly visualized. In this case (lateral side) , the microscopic monitoring of the effectiveness of the cut , depth of the section and extension of the area deprived of periosteum can be performed. It is obvious that this form of control is not feasible on the medial surface of the operatory region.

The consequence of this circumstantial limitation is twofold. First, as it has been suggested, it is not possible to document the extension of the periostomy, neither can be guaranteed in an absolute fashion the effectiveness of the surgical cut on the medial surface of the condyle at the moment of the intervention. Although, the testing conducted during the pilot phase, enable us to affirm that the surgical technique was largely adequate to ensure the experimentally induced discontinuity of the periosteum on both sides of the condylar process.

Moreover, the anatomic proximity of the lateral pterigoyd muscle, and more concretely, the inferior limit of its insertion upon the medial surfaceof the condylar process, make difficult the labour of stripping over the medial surface. The risk of superficial dislaceration of the muscular fibers of the inferomedial stratus of the lateral pterigoyd muscle is a fact to bear in mind. The deep affectation of the muscular process, occasionally followed by focal necrosis, has been previously reported in the literature (Koski and Ronning, 1974).

The implicit limitations to this surgical modality as well as the technical difficulties previously expressed, made it impossible to exclude, in an absolute way, the likelihood of appearance of some of these secondary effects.

Given the limited scope and procedural constrictions of this study, the potsurgical confirmationof integrity of these structures was not performed. If, in reality, the muscular process was injured, that could have affected the results to an unknown extent. The reliability of the procedure necessarily lies on the level of technical refinement that was accomplished during numerous pilot surgeries in which no injury of the periostem was found upon inmediate sacrifice of the animals. Likewise, the accuracy of the surgical technique becomes of crucial importance. It was found necessary the use of a standarized technique, sufficiently consistent in all cases. Again, the fiability of our methodology rests on the level of precision accomplished in the pilot phase.

The microscopic monitoring, when available like on the lateral side, provided inestimable help in order to control the level, depth and extension of the periosteal resection. This allowed the consistency of the surgical procedure from one animal to another, as well as the accuracy on every case.

Some previous studies emphasize the presence of an important inflammatory infiltrate of the medular component, during the inmediate potsurgical period (Koski and Ronning, 1974, 1984, 1985). In some isolated case, a situation that has been defined as "shock reaction of the first day", has shaded the validity of the histological results obtained in the days inmediately following the surgery.

This intense inflammatory infiltrate exhibits clear predominance of eritrocytes in the marrow space, with noticeable displacement of any other cell lineage. It has, nonetheless, transitory character. In fact, it undergoes a rapid evolution towards normality, with restablishment of the cellular balance in the marrow space and initiation of the process of histological regeneration of the periosteum towards the third day of the potsurgical period.

In fact, in previous reports by Koski and Ronning (1975, 1983, 1985), evidence has been provided regarding the healing process taking place in the periostomized surgical site and surrounding structures after the operation. Subsequent surveys permitted the authors (koski and Ronning, 1985) to document that the healing process was practically completed on the tenth day on the large majority of specimens.

The possibility of injuring the muscle has already been mentioned in previous comments. In most cases, when a careful technique is performed, the traumatic dislaceration of the lateral pterygoid muscle was of minor degree and transitory nature (koski and Ronning, 1985). In some isolated case, once again, the damage was important and a focal necrosis of irreversible characteristics was present. Reversible or not, the importance of this event in the

outcome of the surgical intervention is unknown, very specially during the first phase of the experimental period.

It is not a purpose of this study to evaluate the effects of the periostomy during the inmediate potsurgical phase (first 48 to 72 hours after the operation). Therefore, provided that the reaction is of reversible and transitory nature and does not seem to affect the ulterior process of regeneration, it was not found imprescindible to monitor the presence of this phenomena.

The use of an experimental surgical procedure as a methodological element in a project of investigation, conveys a series of limitations inherent to the technique itself. So, it is pertinent to mention that the alteration of the biological environment of a given anatomic region or functional unit, unavoidably implies a rupture of the physiological balance in wich the observed apparatus normally develops its activity.

It becomes, then, a basic requirement of any experimental model, to introduce an alteration highly selective upon a controlled number of the possible paramenters or variables implicated in a given function. This would allow to interpret the results as a direct outcome of our experimental manipulation upon the variable subject of study.

This sort of methodological approach is not feasible, unfortunately, in a surgical experimental procedure. It turns out to be so, even in the best of the possible attempts and although every effort is made to operate in a non traumatic way and to execute a selective manipulation of the structures object of study. Any surgical intervention involves an alteration of several biological factors implicated in the biofunctional balance that is proper of any articular region.

Furthermore, even if a highly refined technique is utilized and a careful management of the surgical field is achieved, is not possible in conclusion to avoid the sequence of undesireble secondary effects that follow any surgical trauma. Consequently, the validity of

our conclusions has to be interpreted under the light of the limitations inherent to any study that requires the use of methodology of this nature.

Some assumptions are neccesarily made when we try to define the very nature of some of the parameters involved in the general process of facial development. In order to be able to validate the results and conclusions of our study, we should be able to establish a qualitative and quantitative correlation between the mechanical stimulation generated by muscular and tendinous structures attached to the periosteal membrane. We ought to be able to characterize the nature, quantity and modality of the mechanical stimulation generated by the physiological activity of these structures attached to the periosteal membrane and the biological response that can be observed in different areas of the body surface.

A methodology has not been developed to the sufficent degree of finesse as to be able to define the nature and quantify the amount of mechanical stimuli generated by these attachments without altering the physical properties and, therefore, biological *"status quo"* of the periosteal membrane.

There is an acceptable degree of scientific evidence that a correlation exists between the mechanical stimulation generated by the physiological activity of musculotendinous structures directly involved in masticatory function and the type of biological response observed in the long bone surface, in terms or remodeling, aposition and resorption. However, as reported by Enlow (1990) there is no exact topographic correlation between the areas of muscle insertion or pull and the remodeling activity in the bone surfaces of the craniofacial skeleton. Other studies (Hylander and Bouvier, 1979, 1980; Oyen,1988) provide further evidence that correlations can be established between the amount of forces generated by the masticatory muscles and the level of mechanical stress (tension-compression) that can be associated in bony surfaces, related or not to the area of attachment.

For the above reasons, we do propose, according to the results obtained and the experimental model under investigation in this study, that a correlation exists between the muscular and tendinous structures attached upon the periosteal membrane and the tension generated on the periosteum. Furthermore, it is a premise and a conclusion of this study, that the tension generated by these structures bears a biological correlation with the histological response observed in specific loci of the craniofacial complex. As to the mandible in particular, the contribution of the periosteum to growth activity in the condylar cartilage has been proposed to be both histological and biomechanical. Probably, both phenomena are but distinct manifestations of the same basic process of control, whose intimate nature we are trying to approximate with studies like this. A better understanding of the role played by the periosteum in growth and development of the mandible, will facilitate our undertanding of the mechanisms of action of functional appliances. From this point of view, a great improvement in the effectiveness and clinical aplications of the functional appliances would be accomplished if the sequence of biomechanical stimuli is fully understood. If the periosteum can be confirmed as playing a role of linking factor or regulatory element in growth of the ramus and its condylar cartilage, our ability to manipulate the biophysical conditions or the biological *status quo* of the periosteal envelope will enable us to induce controlled changes in growth activity.

The surgical approach was used in this study to manipulate the biological environment of the periosteum. The surgical periostomy produced clearly observable changes in the growth parameters estimated in this work. The possibility of clinically use a surgical intervention upon the periosteal membrane in order to introduce a change in the growth process, will require a better understanding of the mechanisms of action of the periosteum. Future studies should be conducted in order to elucidate the effect of surgical manipulation of the periosteum in long bones as weell as in the craniofacial complex. If the

effects of such a surgical procedure can be fully characterized and perfectly understood, it would be possible to determine proper indications and adequate performance for its clinical use. It must be considered that if the periostomy might affect growth by producing an increase or a decrease of the growth activity. In one case or the other, provided the results are predictable and controlled, the clinical aplication of the procedure would be possible since there are clinical situations of mandibular prognathism or mandibular retrognathia. The effects of the surgical periostomy both during the disrupted-periosteum phase and during the phase of healed-periosteum must be reported, as we have attempted in the present work. Moreover, the long-term effects of the intervention should be characterized before the procedure is clinically used.

The complete characterization of the role of the periosteum in the growth process of the mandible has not been accomplished, although its relevance in the growth of the condylar process and its cartilage has been previously suggested. The present study provides some further evidence supporting the notion that the periosteum plays an important role in the activity and regulation of the growth process under physiologic and experimentally induced conditions. Such a role targets in some of the more exciting and enigmatic problems of craniofacial growth. Future research must be conducted in order to improve our understanding of the control mechanisms involved in craniofacial development and the importance and contribution of the periosteal membrane in bone biology.

SUMMARY

The present investigation was conducted in order to evaluate the role played by the periosteum in regulating growth of the mandible and its condylar process. The experimental procedures used in this study in order to achieve the purpose just described can be summarized as follows. A bilateral surgical periostomy around the neck of the condylar process was conducted in normal growing 21-day-old rats and a hyperpropulsion device was bonded onto the upper incisors in order to achieve an anterior repositioning of the mandible of 2 mm of sagittal advancement and a bite opening of 1 mm., in the group of combined treatment (group I). The two procedures (surgery and appliance) were combined in order to determine whether the experimental manipulation of the periosteal envelope surrounding the mandibular condylar process modifies in a significant manner growth in animals undergoing experimental mandibular hyperpropulsion. In the second group (group II) only the surgical procedure was performed. In the next group (III) only the hyperpropulsion device was utilized and a bilateral sham operation was done. The effect of the device on growth of the mandible and its condylar cartilage was evaluated. A fourth group (Group IV) in which only a bilateral sham surgery was performed, served as control.

In each animal, a quantitative evaluation of the dimensional features of the layers forming thecondylar cartilage was performed. Individual measurements of the linear height i.e. thickness as well as an area analysis and cell counts were carried out for every layer as reported in the Materials and Methods section. The results obtained through use of these mnethods is summarized below. At the end of the first phase of the experimental period (first two weeks), comparisons between the values obtained in the dimensional analysis of the growth zone and hypertrophic zone for the different groups did not show statistically significant differences (Tables IA-1, IA-2, IC-1, IC-2). The dimensional values obtained for the

functional chondroblast zone and for the measurement of the total height of the cartilage, indicated greatest figures for the surgical groups and lesser for group III, the control presenting values in between the previous ones (Tables IB-1, IB-2, ID-1, ID-2).

The analysis of cellularity at the end of the first phase of the experimental period indicated that the control group had the greatest cell counts in the growth zone and functional chondroblast zone. Differences for the mean values of cell counts in the hypertrophic zone of the groups were not statistically significant. The surgery-only group presented the lowest values and the two groups undergoing mandibular hyperpropulsion (groups I and III) exhibit values between the control group and the surgery-only group (group II) (Tables II from A to D; Figs II from A to D). The total cellularity analysis (including all layers in the counting) presented similar data, indicating a very high level of significance in the differences between the mean values of the control group and any other one, specially the surgery-only group.

At the end of the second and final phase of the experiment (five weeks), the dimensional values for the growth zone and the functional chondroblast zone provided a similar order of the groups: the greater values corresponded to group III, followed by the control group (IV) and the surgical groups (I and II) presented the lowest values. The total measurements of dimensions presented similar values. The differences for the hypertrophic zone were not significant.

The cell counts at the end of the second phase indicated that all experimental groups had greatest values than controls in the growth zone; the surgical groups exhibited higher figures than group III. In the functional chondroblast zone, the differences were not significant, as they were not significant for the hypertrophic zone either. The total cellularity counts exhibited similar values to the ones presented for the growth zone (Tables IV D-1 and IV D-2).

Similarly, a two-way analysis of variance was carried out in order to determine the interaction of the factor PHASE and the factor GROUP (experimental procedure) for each layer in both the thickness measurements and the cell counts. The results of this two-way ANOVA indicated a very statistically significant level of interaction, showing that the experimental procedures affected the cartilage differently in the first and in the second phase of the experiment.

The results of our study indicate that the surgical manipulation of the periosteum induced a significant change in the growth parameters evaluated in animals under physiologic or experimental conditions. Therefore, it is conceivable to support the notion that a correlation exists between the periosteal and the cartilaginous component in mandibular growth. We propose, in agreement with some other authors (Koski and Ronning, 1975, 1985; Petrovic, 1981, 1982) that the separation between the cartilaginous and the periosteal component in the mechanisms of mandibular growth is very questionable. The results of our investigation support our argument that periosteal integrity and periosteal tensions operate as one of the regulatory components of growth activity present in the mandibular condyle and in the condylar cartilage. The intimate mechanisms by which biomechanical stimuli or factors are transformed into a histological response continue to be of great interest and controversy. Future efforts in this line of investigation could focus in the elucidation of such intimate mechanisms, which would improve substantially our understanding of the mechanism of action of functional appliances and mandibular growth. Likewise, future research should undertake the task of fully characterizing the integrative role played by the periosteum in the regulation of mandibular growth. The complete characterization of such a role should include the understanding of its biomechanical action in its histological contribution. The evaluation of aposition-resorption activity throughout the condylar neck as well as in different locations of the mandibular structure was not carried out in the present study given the limitations of this

project. Nevertheless, such an information should be of great value in order to understand the effects of our experimental manipulation in the overall process of mandibular growth, and could be the subject of future research.

Finally , it is of crucial importance to get to know the nexus that is established at a cellular level as well as the system of stimuli transmission, registration, integration and control of the overall proces and final outcome. In this process, a first approximation could be provided by elucidating the role of the periosteum in mandibular growth. The following step consists in documenting the transmission of growth messages and their integration whithin the target cell. The intimate mechanism by which diverse factors of different nature interact and provide information that is processed at a cellular level. The sequence of events leading to the generation of an adequate response, and the control mechanism by which the outcome of the process is modulated to provide morphofunctional balance within the craniofacial complex, would be our next and ultimate objective in the understanding of growth and development.

CONCLUSIONS

1-The periosteum acts as an indirect local controller of the process of growth in the condylar cartilage under the experimental conditions used in the present study.

2-Experimental manipulation of the integrity of the periosteum or modification of its physical, biological or environmental conditions, can induce a modification in the rate of growth of the cartilage of the manbdibular condyle.

3-The nature of the control mechanism by which the periosteum exerts an influence in the growth of the condylar cartilage could be either mechanical (biophysical), citohistological (histodifferentiation of skeletoblasts) or both.

4-The results of our study allow us to speculate that the alteration of the mechanical environment (or circumstances) affecting the biophysical conditions of the periosteum carry out a modification in the growth rate of the condylar cartilage.

5-Surgical manipulation of the continuity of the periosteum carry out a decrease in cerllularity in the condylar cartilage, although the dimensional features do not seem to be affected in the same way. Upon healing of the periosteum, an important increase of the growth rate is detected.

6-A decrease in the tension generated upon the periosteum by capsular and capsular attachments by means of surgical periostomy carried out a severe drop (or significant) of growth activity understood in terms of cellularity, in the condylar cartilage.

7-When the tension of the periosteum presumebly regains normal values after the healing process of the periosteum is complete, the rate of growth noticeably increases as to overpass the values observed in surgical groups.

8-A hyperpropulsive appliance, worn on a full-time basis induces an effect of transitory fall of growth activity, its rate being significantly less than in controls regarding both cellularity

present in the condylar cartilage as well as dimensional features in the different layers. This transitory effect is followed by an increase of growth activity in the condylar cartilage that is observed at the end of the experiment, where all parameters evaluated were greater for this group than for controls.

9-It can be confirmed from our results, that mandibular hyperpropulsion, when properly performed by means of appliances not preventin a physiologic range of movements of the mandible, induces an increase of growth of the mandibular condyle.

10-In animals undergoing combined treatment, the release in tension of the periosteum after the surgical intervention resulted in a decrease of cellularity of the mandibular condyle. Upon healing, a significant increase can be detected. Thus, it can be concluded that during the phase of disrupted periosteum, neither the physiologic tension of the periosteum was present, nor the increase in tensional levels generated by all menisco-capsular attachments could be operative upon the periosteal surface. The tension of the periosteum was prevented, as a result of our surgical procedures,to exert its role of controller of growth activity. When histological and physiological integrity was presumebly restablished after the healing was completed, an increased tension of the periosteum generated by the protrusive position of the appliance and transmitted by all menisco-capsular attachments as well as muscle insertions, resulted in an increased rate of growth of the condylar cartilage.

12-It can be speculated that the rebound effect in hte growth activity after the healing process is completed, is responsible for the difference in growth activity between these animals and the group of appliance wearing only.

13-The hyperpropulsor appliance does not provide any further growth in those animals undergoing simultaneously periostomy. In conclusion, the appliance does not succeed to provide a significant further increase of growth relative to the increment related to the healing.

14-The periosteal healing carried out a rebound effect in the growth activity sufficient to mask and overcome the effect of the appliance in growth stimulation.

15-The traumatic component involved in any experimental manipulation generates a decrease in the growth rate, as expressed by the fact that all experimental groups showed a lower rate of growth relative to the control group. When the traumatic component disappears, the effect of the experimental procedure can be observed.

16-The fact that the experimental manipulation of the periosteum induces changes in the activity of the condylar cartilage supports the notion that a correlation exists between the two components of mandibular growth. The effect of our experimental manipulation in the growth activity taking place in other locations of the mandibular structure was not evluated, although such an information would most certainly be revealing in our undertanding of the local control mechanisms of mandibular growth.

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