UNIVERSITY OF OREGON DENTAL SCHOOL

METABOLIC AND DIMENSIONAL CHANGES IN THE PERIODONTIUM OF RATS INCIDENT TO TOOTH MOVEMENT

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CHAPTER I

INTRODUCTION

All banded orthodontic appliances accomplish their purposes by delivering forces to the periodontium through the banded teeth. The introduction of these forces alters the pre-existing biological equilibrium and initiates changes which result in movement of the teeth toward new locations at which they would again be at equilibrium with the forces impinging upon them.

At first glance, it would appear that the teeth are simply "driven" through the bone by the mechanical load. Such indeed was the belief of many practitioners before the turn of the century. The teeth were perceived as rigid bodies which, under load, pushed their way through the relatively plastic bone much as an upright post can be pushed through sand. The logical consequence of this position was the belief that the more force the clinician used, limited only by the patient's physical tolerance, the more rapidly and satisfactorily would treatment goals be achieved. Clinical orthodontics thus tended to become primarily an exercise in engineering maximal force application.

To thoughtful investigators, however, it was early apparent that profoundly important biological processes

intermediated between the first cause (mechanical load) and the final effect (tooth movement). Thus, F. Noyes later stated, "It seems axiomatic . . . that mechanical forces applied to the teeth are not used to produce mechanical movement, but are used as biological stimuli to set up vital reactions in the tissues." (59) The logical consequence of this position was the realization that the optimum force for the induction of tooth movement might be far below the maximum force deliverable, and that heavy forces might themselves reduce the efficiency of tooth movement by disordering biological processes.

Thus the exact pattern of biological responses which follows the delivery of a force to a tooth became a prime theoretical concern of the practicing clinical orthodontist. Indeed, the investigation of the manner in which mechanical forces delivered to teeth are translated into biological instructions to alter the anatomy of the dental unit continues to be one of the most intriguing and compelling tasks in orthodontics. The present study is intended as a contribution to the process of this investigation.

The currently dominant hypotheses on the nature of tissue changes in tooth movement were generated primarily by Sandstedt, Oppenheim, and Schwarz during the first thirty years of this century. Their findings were based on careful examination of tissues taken from a small number of conventionally prepared specimens using classical histological

techniques. Reflecting the general physiologic theory of the period, much emphasis was placed on questions of vascularization and cell morphology. The conventionally accepted schema which emerged as a result of the work of these men and their associates may be summarized as follows:

The effect of the application of biologically acceptable force levels is to move the tooth within the periodontal space, narrowing that space on the "pressure" side and widening it on the "tension" side. The vascular bed on the "pressure" side tends to be collapsed, thus inhibiting blood flow. It is felt that the "stretching" of the periodontal fibers on the "tension" side constitutes a stimulus to new bone formation, while the reduction in blood supply on the "pressure" side creates conditions for bone resorption.

In terms of tissue modification, the early consequences of force application on the "tension" side are said to be rapid stimulation of new fiber production and greatly increased cell proliferation. On the "pressure" side, there is held to be an early tendency toward reduction in cellular elements (due to the pressure-produced inhibition of blood flow) with some disorganization and possibly some reduction in fibrous elements.

During the past fifteen years, new instrumentation and new knowledge from the field of molecular biology have made it possible to attempt to test the validity of these classical histologic findings by other means. It has been

established that the production of protein in tissues is the sequential process whose pattern is determined and controlled by the organism's genetic material. (15) For each specific protein product of cellular metabolism, whether structural or enzymic, there exists in the nucleus a specific segment of desoxyribonucleic acid. This DNA codes for the production of a complimentary messenger ribonucleic acid (mRNA). The code thus incorporated into the mRNA in turn determines the specificity of the protein by dictating the kind, order and number of amino acids from which the protein is assembled. The events of protein production occur in a specific temporal sequence. First the appropriate DNA is activated. Then new RNA's are formed. Finally a protein is assembled. The rates of formation of new DNA, RNA and protein may be monitored by measuring the rates of incorporation of various tritiated metabolites. Tritiated thymidine will be incorporated only into DNA. (1, 48) Tritiated uridine will be incorporated only into RNA. (27) Tritiated proline will be incorporated primarily into collagen. (14)

This new knowledge of the general pattern of connective tissue formation gives us an excellent means of testing hypotheses as to the nature of tissue changes incident to tooth movement. To the degree that the classical description of tooth movement is correct, we would have reason to expect the following changes in the rates of incorporation of thymidine, uridine, and proline during the first seventy-two hours

of tooth movement when compared to untreated control animals. On the "tension" side all three rates would be expected to increase as a consequence of generally increased anabolic activity. On the "pressure" side, the thymidine and proline rates would be expected to decrease, reflecting lowered cell and fiber production, while the uridine rate would be less predictable due to its possible utilization in RNA synthesized to participate in catabolic reactions. Areas surrounding untreated contra-lateral control teeth in the experimental animal would be expected to show minimal changes.

The presently reported experiments involved attempts at such hypothesis testing by the study of metabolic and dimensional changes in the periodontium of rats subsequent to the induction of tooth movement by the unilateral placement of elastic wedges between the upper molars. Alterations in the pattern of cellular activity were monitored autoradiographically following the injection of suitable tritium labeled metabolites. An attempt was made to employ sample sizes sufficiently large so as to yield statistical rather than descriptive results.

CHAPTER II

REVIEW OF THE LITERATURE ON TOOTH MOVEMENT

It is usually said that research in tooth movement began with the work of Sandstedt, which was first published in 1905. What is not so widely realized is the fact that many years of critical observation and discussion preceded the experimental stage of tooth movement research. This phenomenon is common to most developing scientific disciplines and, as in most disciplines, the biases of the observational stage of orthodontic research tended to define the first experimental problems and to distort the conclusions drawn from them.

Examination of the early orthodontic literature reveals that each of the four fundamental bio-mechanical problems which have concerned experimentalists since Sandstedt and Oppneheim had already been considered and debated extensively prior to 1890. (23, pp. 44, 146) These problems are:

1. What is the nature of the tissue changes in tooth movement? (What are the anatomical locations of the changes and what are the significant biological phenomena which characterize them?)

2. Can we define permissible and appropriate force magnitudes and speed of movement in clinical orthodontics?

3. Should intermittent or continuous forces be used?

4. What is the location of the axis of rotation of the tooth under load?

Consideration will reveal that the last three questions are in fact special aspects of the first in the sense that a complete understanding of the nature of tissue changes in the periodontium under load would immediately dictate solutions to the other questions. This present study deals primarily with the first problem. An attempt will be made, however, to point out some of the consequences of hypotheses on tooth movement as they affect the other three problems cited.

The point of departure of both Sandstedt and Oppenheim was the less well known work of the French physiologist, Flourens, who in 1841 published a monograph on the formation of teeth and bones. (25) In it he discussed his own work with vital staining, and reviewed the earlier experiments with madder by Belchier, Duhamel and John Hunter. Flourens drew the amazingly perceptive conclusion that mature bone, rather than being a stable and unaltering substance as had previously been supposed, was a material in a continual process of renewal by resorption and deposition. His predecessors had concerned themselves primarily with the growth and development of bone. Flourens examined relatively mature bone and was first to perceive the turnover process which we now know characterizes the metabolism of this tissue throughout life.

It appears that Flourens believed that the resorption of bone in the eruption of teeth was caused by pressure from the underlying tooth bud. In any event, this idea came into the literature soon following his work and is associated with his name by Oppenheim and Schwarz. It was a single logical step from such a conception to the idea of pressure-induced resorption and tension-induced deposition as accommodations to the application of exogenous forces by orthodontists.

G. V. Black's observations supporting this belief were quoted by J. N. Farrar in 1888. (23)

> In his valuable work "Periosteum and Periodontal Membrane," Dr. Black says, 'When teeth are undergoing changes of position, changes in the attachment in the principal fibers of the peridental membrane occur, which are caused by adsorption and rebuilding of bone. Indeed,' says Dr. Black, 'these fibers seem to disappear with the appearance of the osteoclasts, but wherever the bone is not covered by these cells, the fibers are found to be in position. At 'E,' (referring to a figure) a portion of new bone has been built on to the old, in which the ends of the fibers are secured. In this way, it seems, adsorptions and changes in the alveolus may occur slowly, or even with considerable rapidity, and sufficient attachment of the principal fibers of the membrane be maintained to hold the tooth securely while its position is being changed. Parts of the fibers of the membrane, appear to be cut away and some portions of the bone removed, then the fibers re-form and re-build into the wall of the alveolus by a new deposit of bone about their ends. These changes are not confined to young animals or young persons, but may be found in progress in the old, though generally more irregular. . . . I have not had the opportunity of examining a case in which the artificial movement of the teeth, as in the correction of irregularities, has been made, but from what I have seen I suppose that the absorption and rebuilding occur in precisely the same way.' (My emphasis. S.B.)

The experimental work of Sandstedt (101) was taken as an affirmation of the appropriateness of the principle of Flourens in explaining the nature of tissue changes in the alveolus when a tooth is moved. Sandstedt's experiment is described by A. M. Schwarz. (76)

> A labial arch was placed on the six maxillary incisors of a dog. Bands were placed on the canines with small horizontal tubes in which the arch was fixed. Distally to the tubes were screws drawing the arch backward. Thus, the arch pressed the incisors lingually, while the canines were drawn mesially. This experiment lasted 3 weeks. During this time the crown of the central incisors were moved about 3mm lingually. . . .
>
> The results of Sandstedt's investigations are as follows:
>
> 1. On the side of pull, with both weak and strong forces, a deposition of bone takes place on the old alveolar wall. The newly formed bone spicules follow the direction of the strained peridental fibers. One can distinguish precisely the limit between the newly formed bone and the old alveolar bone; the latter is not changed.
>
> 2. On the side of pressure, the old alveolar bone is equally resorbed by weak forces. The surface of the tooth itself remains intact. By these fundamental findings, Sandstedt was the first to ratify the pressure theory of Flourens, also for the orthodontic movement of teeth. (My emphasis. S.B.)
>
> 3. By strong forces the peridental soft tissue is compressed at first on the side of pressure and cannot resorb the old alveolar bone, because it is deprived of its vitality. Instead of this, an active resorption soon begins in the neighboring marrow spaces of the alveolar bone; so the bone and the compressed soft tissue in the region of the greatest pressure are removed. When all the necrotic material is removed, the tooth assumes at one pull a new position. Sandstedt calls this process the undermining resorption. This very interesting biologic reaction of the alveolar bone explains the fact that by the use of strong forces orthodontic results also are obtained.

The celebrated work of Oppenheim was first published

in 1911. (61) Oppenheim's evaluation of the impact of his paper on orthodontic practice was quite correct if a trifle less than modest. "My first experimental studies on the teeth of monkeys, and the conclusion drawn from these experiments," he later wrote," have, for many years, been the guiding principle of orthodontic practice. They constituted a turning point from the use of strong forces toward the application of gentle forces." (60)

Oppenheim's original observations were made on the deciduous teeth of a single baboon. The portion of his observations which has had impact on succeeding workers concerns itself exclusively with tissue changes in the immediate area of the alveolus. Oppenheim stated,

> My findings were not identical either with the elasticity theory of Kingsley-Walkhoff, or with the pressure theory of Flouren (sic) which postulate resorption on the side of pressure, and apposition on the side relieved from pressure.
>
> Some very interesting findings in which I found an analogy in conditions present in monkeys at the time of tooth eruption, induced me to the belief that this analogy in the findings was a proof for the fact that the forces used in my own experiments were of the proper intensity. I accepted the transformation theory of Wolff, because of the transformation which again occurred during the period of retention.
>
> In my above mentioned article, I stated that the process observed constitutes a classic example of the change from compact bone into trabecular bone, and I have defined it in the following way: 'The bone tissue, compact as well as trabecular bone, reacts upon pressure with the transformation of its whole architecture. This takes place through resorption of the present bone and apposition of new bone. Both processes start simultaneously.' In this original statement, which was made more than 20 years ago,

> nothing needs to be changed, and also the histologic findings remain in force.
>
> On the basis of new experiments and contributions of other authors during the last years, I can confirm the fact that resorption and appositions are the only two factors which produce changes in the bone tissue (my emphasis. S.B.); this statement is also borne out of the laws of general bone pathology. In this view I was strengthened by the fact that the regressive changes occurring during retention and ending with the appearance of new lamellar bone, are taking place along the same lines; therefore, I came to the conclusion that either previously formed bone is being transformed, or newly formed bone is being adjusted to the functional demands. The functional adaptation of the bone tissue is, during active treatment, induced by external stimulations. The histologic pictures disclose that the new arrangement of the bone trabecular and of the periodontal fibers represents the best functional adaptation to these stimulations.
>
> The terms 'stable' and 'normal' should not be applied to the form of the tissues themselves, but to the potential qualities of the tissues to assume, according to certain stimulations, different forms.
>
> The transformation of the bone could also in my experiment, as I have already stressed, only occur through resorption and apposition; without Flouren's pressure theory, there can be no bone change, no bone transformation. (My emphasis. S.B.) (61)

Oppenheim's theory of tooth movement, then, has two components: the well-known transformation theory of Wolff, first published in 1884, and the turnover theory of Flourens', circa 1841. But he appears to misread Flourens in the sense of believing that pressure is a precondition for bone turnover. This Flourens did not believe. Indeed, his main emphasis was on the fact that turnover was a normal and continuous phenomenon in all bone.

"The mechanism of bone development," Flourens stated, is obviously a continuous mutation of each component of the bone. The bone I observe does not now have any particle it

had a while ago, and soon will not have any of today's particles. During this continuous change in material, the shape stays very much the same. This is one of the primary and basic laws in organisms. In everything alive, the form is more persistent than the particulate content." (25)

Flourens then quotes Cuvier, who said,

> In living organisms, no molecule stays at the same place, all are moving in and out. Life is a perpetual swirl with a complicated but constant purpose, as are the participant shapes but not the individual molecules themselves. To the contrary, the actual content of a living body will soon no longer be here, but the organism does have the capacity to draw to itself the materials which will comprise it in the future in such a way that its shape will continue unchanged. The form of living bodies, then, is more essential than the particulate materials comprising them, because the form remains unchanged. (25)

And Flourens concludes, "We can say then, that the idea of continuous change, for Cuvier the conclusion of philosophic meditation, became a reality in my experiments with madder." (25)

With regard to the pre-conditions for bone turnover, modern isotope studies would hold with Flourens that pressure is not required. Whether pressure accelerates or retards turnover time is a different question which is yet to be studied.

In the period following the publication of Oppenheim's work there tended to be a polarization of researchers on tooth movement tissue changes into two camps. One group took

the work of Sandstedt as its starting point. The most vocal spokesman of this group was A. M. Schwarz, with whom were associated B. Orban and B. Gottlieb. The other group was headed by Oppenheim and included Grunberg and the members of the Angle school. Certainly there was a good deal of personal acrimony in the disputes, and at times the distinctions between the positions of the partisans of the two sides became rather miniscule, but it is perhaps helpful to review the substantive positions of the two camps in terms of the four questions raised above.

1. On the nature of tissue changes in tooth movement.--In this area the two groups started from the same assumption that the locus of tooth movement was entirely within the periodontal space. The force of the appliance was held to displace the tooth within the PDL space. Excessive force would express fluids from the PDL and bring the tooth into contact with the unyielding alveolar bone producing root resorption and other pathologic changes to the tooth and paradontium.

Neither group accepted the possibility of deflection of the bone as a component of the tooth moving process. In all probability, both groups reasoned on an a priori basis that any force sufficient to deflect bone would inevitably produce gross pathology. Both groups thus rejected what was called the elasticity or "bone bending" theory of Kingsley and Farrar.

It is important to note that neither Kingsley nor Farrar maintained that bone bending was the only factor in tooth movement. (23, 44) Both recognized that resorption and apposition occurred within the PDL space. However, both felt that there existed an additional factor of gross flexion of the alveolar process, as indeed there must have been under the force levels of the jack screws then employed for individual tooth movement. For example, Farrar wrote,

> Teeth move by one of two kinds of Tissue Changes in the Alveolus. The necessary changes in their arrangements are accomplished in two ways: by the reduction of the alveolus through what is called absorption on one side of the tooth, followed by the growth of new supporting tissue on the other; and by bending the alveolar tissue. (23, p. 168)

And further,

> Several of the following chapters will be devoted to the discussion, in detail, of these principles, supported by the results of experiments, which point out the most important phases of the two characters of force. First will be considered the theory and practice of moving teeth by absorption of the alveolus; second, the theory and practice of performing operations, by taking advantage of alveolar flexibility, a demonstration that both processes may be made to harmonize under the same law. (23, pp. 142-143)

From a clinical point of view, the position of Oppenheim and Schwarz for light forces and against "therapeutic" utilization of alveolar bending was an enormous advance. As we will attempt to demonstrate later in this paper, however, their rejection of the possibility that phenomena other than tooth movement within the PDL might occur following force application was probably unfortunate from a

heuristic point of view.

In any event the possibility that bone deflection might be a characteristic phenomenon of orthodontic treatment continued to reappear in the literature. The German researcher Wolkhoff believed that elastic deformation in the bone due to residual force effects produced post-treatment relapse by causing "rebound" following appliance removal. This theory was the basis for Skogsborg's Septotomy treatment. (60) Reitan proposes a rather similar rationale today with regard to soft tissue when he suggests severing supracrestal fibers to prevent relapse of rotations. (65)

2. Permissible and appropriate magnitudes of force and speed of movement.--Schwarz divided tooth movement forces into four categories in terms of their purported biological effects. He described them as follows (76):

> First Degree of Biologic Effect.--The force is of such a short duration or so slight that no reaction whatever is caused in the periodontium.
>
> Second Degree of Biologic Effect.--The force is gentle, speaking biologically; it remains below the pressure in the blood capillaries, i.e. less than 20 to 26 grams for 1 sq. cm. surface, but it nevertheless is so intensive that a continuous more or less lively resorption takes place in the alveolar bone at the regions of pressure.
>
> Biologic effect after the force ceases: Anatomic and functional restitutio ad integrum of periodontium and alveolar bone. No resorption of the root.
>
> Third Degree of Biologic Effect.--The force is fairly strong. It just represses the pressure in the blood capillaries of the regions of pressure. At these areas bloodlessness of the tissues takes place, and also suffocation of the strangled periodontium. In consequence of this, resorption of the necrotic tissue parts occurs beginning around the areas of

> pressure. This resorption takes an impetuous course and attacks also those parts of the surface of the root the vitality of which may be injured by the pressure.
>
> Biologic effect after the force ceases: Functional restitutio ad integrum of the periodontium, alveolar bone and the resorbed surface of the root, but perhaps also progressing resorption of the dentine.
>
> Fourth Degree of Biologic Effect.--The force is strong; it squeezes the strangled periodontium on the side of pressure. At the strongest degree the tooth touches the bone after the soft tissues are crushed. Resorption occurs around the regions of pressure, and also undermining resorption from the marrow spaces. There is danger that the injured tooth surface may be resorbed.
>
> Biologic effect after the force ceases: The same as that of the third degree, but besides the danger of pulp death as a consequence of tear in the tissues, there is crushing or bleeding of the apex and possibility of ankylosis of the tooth with the alveolar bone.
>
> This diagrammatic division teaches us that in practice continuous forces of not more than 15 to 20 grams for 1 sq. cm. surface, shall be used to keep safely within the limits of the biologic optimum. (My emphasis. S.B.)

Oppenheim also argued for light forces but felt that varying situations made prescriptions of precise force value less than meaningful. He stated that, "after all, firmness and painlessness are the only clinical criteria of properly adjusted forces," (60, p. 141) and pointed out that precise quantitation of treatment forces as Schwarz proposed was in any event a technical impossibility. Incidentally, Farrar had earlier used the criterion of freedom from pain (though not the criterion of firmness) as a force limiting factor. However, he had felt it was possible to move a tooth 1/240 of an inch a day with impunity. (23, p. 158) This rate is

equivalent to closing an 8mm bicuspid space in 90 days, a pace which most modern orthodontists would consider excessively rapid.

3. Intermittent vs. Continuous Forces.--Oppenheim contended that intermittent forces should be used allowing time for rest periods between adjustments. (56, p. 254) In this regard he reflected the earlier position of Farrar, who contended that all physiologic processes reflected what he called "The Law of Labour and Rest."

> Since fatigue is but a form of pain, it also may be made to illustrate a phase of the subject. This is exemplified in that every act of life comes within the law of labour and rest. If the arm is extended at right angles with the body, it soon tires and falls. Long-continued movement or work is fatiguing; even standing for a long time is exhausting, and change of position for the muscles is but another form of rest. Organs, as well as muscles, require periods of rest; the eye tires of seeing, the ear of hearing, so also the sense of taste. Nor is this all, for the involuntary muscles require periods of repose. The lungs, or rather the different muscles that perform the act of respiration, rest between their alternating movement; while one set is causing inhalation, the other set rests; but during exhalation these muscles take their turn at work, and the other become passive; so also does the heart rest in the intervals of peristaltic action.
>
> To return to the main subject, we see from these premises or considerations that the differences in operations which bring about healthy or physiological changes by pressure, depend on the character of force applied, including degree and length of time; that the question of pain depends upon controlling the force, applying it lightly if continuously, or periodically if greater; the latter allows the tissues to act, and then grants them a period of rest. (23, p. 160)

The Schwarz-Gottlieb-Orban school, however, felt that

the effect of the remission of force was to allow the formation of a layer of osteoid on the pressure side of the alveolus during the "rest" interval of treatment. They believed that the subsequent movement of the tooth against this osteoid during the next active treatment phase led to root damage. (29) Oppenheim rejected this position, saying,

> It has become a modern slogan in the orthodontic literature that the cementum is in danger of resorption especially when intermittent pressure is applied, through that osteoid tissue which is being formed in the intervals. However, the intermittency or the continuity of the force has nothing whatever to do with it. In both these methods, it is the extent of force alone that is responsible for the root resorptions, if it exceeds the permissible compression of the periodontal membrane. (60)

In the dispute about continuous versus intermittent forces, the partisans of Schwarz and Oppenheim each claimed that Sandstedt's work supported their own position. Sandstedt, it will be remembered, employed an arch which was activated by a screw. Oppenheim quite correctly claimed the force was intermittent. Schwarz, however, contended that Sandstedt "drew the screws only a little each day so that the force used was practically constant." (76, p. 331)

The controversy over continuous versus intermittent forces continues to the present day. In a sense, Burstone's segmented arch and Begg's system represent the Schwarz light continuous force school. Reitan too, seems to reflect this position. On the other hand, the retention of three to four week intervals between adjustments with edgewise arches

following low deflection adjustments represents a continuation of Oppenheim's intermittent force principle. On the balance, the prevailing modern opinion concerns itself more with force magnitude than with intermittency versus continuity.

4. The location of the axis of rotation of the tooth under load.--The precise position of the axis of rotation of teeth under load was of great interest to earlier orthodontists and remains of considerable interest today. Its importance seems to have been lessened by the achievement of progressively more positive methods of tooth control and a greatly increased capacity to achieve bodily movement with modern appliances. However, the problem is still of considerable interest, particularly in analyzing the results of lingual tipping of proclined anteriors and the possible mesial movement of bicuspid buds contained in crypts beneath deciduous molars which are being tipped distally.

To earlier workers, the position of the axis of rotation was of extreme importance because the forces employed throughout treatment were primarily of a tipping variety. The prevailing consensus was that in the post active treatment phase teeth tended to upright themselves over the position in which the apex had been placed by treatment. Given this premise, it followed that the location of the axis of rotation in any plane crestal to the apex would mean that at the close of treatment the apex would have moved in a direction

opposite to that in which the crown had been moved. Sandstedt and the Schwarz group held that this was precisely what happened in treatment. Oppenheim felt his own research showed that while heavy forces would result in unfavorable movement, appropriately light biologic forces would result in movement with the axis of rotation at the apex. He stated,

> The correctness of my opinion can be shown not only by the photomicrographs of this article, but also by a simple experiment. If it is true and we know it is true--that bone reacts to the gentlest force by resorption and thus seems to recede from the force, then the root may be represented by a stick which stands with one end on a table and the other end of which is moved laterally without meeting with any resistance during this movement. Then the lower end of the stick will remain in its place without any deviation in the opposite direction of the upper end. (60, p. 254)

It seems appropriate to point out that the only way the stick (read "tooth") could move laterally "without any resistance during the movement," would be if the air (read "alveolar bone") offered no effective resistance to displacement. Obviously, however, if the tooth moves within the PDL space, the bone does soon offer resistance and its most occlusal point tends to become a fulcrum about which the tooth tends to move. The effect of minimal motion soon brings the tooth apex against the resistance of the apical portion of the bony socket. The resultant of these forces must inevitably produce a tendency towards rotation about an axis somewhere in the root. This position is now quite

generally accepted. (73, p. 88) Discussion still continues, however, as to whether alterations in force load result in shifting of the axis of rotation. The theoretical answer would seem to be that if the tooth moves only within the periodontal space, the axis of rotation must remain constant or nearly so but if forces are employed sufficient to deflect the whole bony process the tooth will translate moving the apparent axis toward the apex.

The partisans of Schwarz and Oppenheim each accused the other of drawing conclusions from insufficient evidence. Schwarz and Gottlieb pointed out that Oppenheim had developed his entire position on the basis of the examination of deciduous teeth from a single baboon. Oppenheim countered that Sandstedt and Orban had worked mostly with dogs whose teeth had been placed in traumatic occlusion using high crowns rather than having been treated with representative orthodontic appliances. (29) In this dispute both parties were correct and in fact the remarkable thing is rather how much was achieved by critical examination of small amounts of material. Inevitably the research of both groups was exclusively descriptive. The facilities and techniques simply did not exist to carry out hypothesis testing experiments at the time their work was done. Such work would have required larger sample sizes and more rigorous statistical and histochemical techniques than were available in the 1920's.

The period between the late 1920's and the early

1950's was primarily an era of debate about the meaning of earlier studies. Few additional theoretical or experimental contributions were made. One of the major issues argued was the propriety of extrapolating findings from animal experiments to the clinical problems of humans. Certainly working directly with humans would provide a more representative experimental field, but ethical management, and sample size problems are great. The use of primates for experimental work seems the next best approach, but unfortunately the cost factors and animal handling problems generate difficulty in obtaining adequate sample sizes.

The largest study to date on initial tooth movement changes in humans is that of K. Reitan. In 1951 he published his paper on "The Initial Tissue Reaction Incident to Orthodontic Tooth Movement." (66) His main concern was with the relative effectiveness of activator-delivered intermittent forces and continuous forces delivered by fixed appliances. In general Reitan's observations tend to mirror those of Schwarz.

In the use of primates, limited contributions have been made by several studies. Breitner (11, 12) reported on a series of animals during the 1940's but the number subjected to any single treatment was small. Marshall (53) conducted a study of tooth movement in Rhesus from 1930-1933 but was able to draw few conclusions.

In 1953, Waldo and Rothblatt (91) published a technique

for the induction of tooth movement in young rats by the interproximal placement of elastic bands. This model is unquestionably further removed from naturalistic study of orthodontic treatment tissue responses than primate work would be. However, the low cost, ease of handling, and relatively low individual variation within inbred strains, appear to make it a good one for preliminary hypothesis testing and for hypothesis generating. The nature of the model is such that significant information can probably be obtained only by statistical treatment of results. Studies by Waldo and Rothblatt, Yen and Rothblatt (98) and Zaki and Van Huysen have established fairly good base line histologic information. Dale and Hunt (18) have published briefly on cell replication rate changes using H3 thymidine as a DNA label.

Perhaps the most important article to have appeared thus far, using the rat as a tooth movement research tool, is that of Macapanpan, Weinmann and Brodie. (51) This well designed experiment established the format for subsequent work with rats and confirmed the utility of the rat as an experimental animal in tooth movement research. Unfortunately no untreated control animals were used and so the possibility of changes on the contra-lateral untreated side of experimental animals could not be tested. Further, the statistical techniques used by Macapanpan and her co-workers were merely enumerative and did not take account of the variations among the animals of the sample. The inferential

value of the study is limited by this fact.

More recently Bien and Ayers (5, 6) have conducted an investigation on the intrusion under load of the upper incisors of rats. The use of this model is unfortunate since, as Eccles has shown (21), the periodontal ligament of the continuously erupting incisors of rodents has a markedly different morphology from that of rat molars or of human teeth. Further, this investigation explores the response of the PDL to a shearing force rather than the compressive force with which orthodontists are customarily concerned.

CHAPTER III

RATIONALE FOR CHOICE OF TEST PARAMETERS

Since the time of Schleiden and Schwann and particularly since the work of Virchow, it has been an axiom of all biological science that the basic organizational unit of life is the cell, and that changes in the physiology of living organisms are affected through changes in the activity of cells. The movement of teeth by dentists involves changes in physiology. Therefore, it is appropriate that we look for contemporaneous changes in the activity of cells.

It seems self-evident that cell activity can produce quantitative physiologic changes via one or both of two mechanisms. Either the rate of activity per cell may remain constant while the number of cells is altered, or the number of cells may remain constant while the rate of activity per cell is altered. Finally, some combination of these two situations may obtain.

Given this premise, the key parameters for measurement of metabolic changes in tooth movement become:

1. the rate of new cell formation, and
2. the general rate of cell metabolic activity.

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parameters for the measurement of metabolic changes in tooth movement. A third parameter, the specific rate of cell elaboration of collagen becomes equally important, since orthodontists are especially concerned with the formation of the structural elements of connective tissues.

The rationale for the choice of procedures for testing these three parameters is considered in Appendix F.

CHAPTER IV

MATERIALS AND METHODS

General

The experimental model chosen for the study of tooth movement is essentially that first used by Waldo (92), Waldo and Rothblatt (93), Yen and Rothblatt (98) and Macapanpan (51), and subsequently used by Crumley (17), Zaki (99), Bernick (80) and others. It involves the induction of movement in the maxillary molar teeth of rats by the wedging action of an elastic band placed between the first and second molars (see diagram 1). The effect of the elastic wedge is to displace the first molar mesially, reversing the normal distal drift tendency of that tooth (79), while accelerating the distal movement of the second and third molars. Three structures are present in the experimental field. These are tooth, bone and periodontal ligament. The implicit mechanical assumption underlying the use of this model has been that the periodontal ligament is dimensionally the most readily alterable of these three while the tooth is markedly the least alterable. Thus the effect of any extrinsic force is to move the tooth within the periodontal space. Previous workers have assumed or deduced that tooth movement in this model is almost entirely tipping rather than translational.

The advantages of this model for the study of tooth movement may be summarized as follows: The subject animals are easily avilable, inexpensive and tractable. In addition, since in-bred strains with known characteristics may be used, individual variation can be minimized. Utilization of the molar teeth, rather than of the incisors, avoids the problems inherent in all work with the continuously erupting anterior teeth of rodents. Finally, after an initial period of operator training, the placement of the tooth movement device is rapid and relatively reliable.

The liabilities of the method include the fact that the elastics tend to be lost as separation of the teeth becomes extreme. This fact limits the maximum possible experimental duration to approximately seven days. (92) In addition, the mechanical load per unit of PDL area is probably in excess of that used in most clinical orthodontic situations. Further, assumptions as to the mechanical performance of the experimental model have never been tested. Finally, there is the general problem of extrapolating findings from rodents to humans.

The two specific areas of the PDL chosen for study are located on either side of the mesial root of the maxillary first molar at or approximating the bony crest (see Figs. 1 and 2, Appendix H). The mesial root was chosen because it was deemed sufficiently distant from the point of insertion of the elastic wedge to be protected from the

primary pathogenic effects of the wedge itself. If we assume a tipping motion of the first molar with an axis of rotation somewhere in the root area, the crestal region on the mesial surface of the root is clearly an area of maximal "pressure" effect while the surface on the distal side of the root just opposite this region should correspondingly be a "tension" area.

In general the present experiment had two aims. The first was the testing of the major biological hypotheses outlined above. As work proceeded, however, it became evident that there were questions concerning both validity and the reliability of the experimental model used. The testing of the model to determine the limits of its propriety as a paradigm for tooth movement study therefore became a second aim of the experiment. Efforts were made to obtain quantitative data on the amount and type of movement induced by the elastic wedge, on the appropriateness of the above noted mechanical assumptions, on the effects if any on the untreated "control" side in the experimental animals, and toward the establishment of base line data in untreated control animals.

The experiment involved three successive Runs, each testing the incorporation of a different tritiated metabolite in the periodontal ligament of 65 to 90 gram Sprague-Dawley rats.

Each Run comprised an experimental group and two control groups.

The experimental group for each Run (Group "X") consisted of 24 animals. Unilateral separation of the upper first and second molars, was affected in the experimental animals by wedging a thickness of Unitek 400-154 3/8" elastic between them. The dimensions of the elastic wall approximated .89 by .65 mm. The right side of each maxilla was termed the treated side and the left side was termed the untreated side. Within each Run, aside from tooth separation itself, the only experimentally introduced variable was the length of time after the induction of tooth movement at which the animal was sacrificed. Two animals were sacrificed every 6 hours from 6 to 72 hours. Two hours before sacrifice each animal received an intraperitoneal injection of an appropriate tritiated metabolite. (The rationale for choosing this 2 hour time interval is elaborated in Appendix F.)

The first control group of each Run (Group "B") constituted a control for background radiation and technique artifact. It consisted of three animals, sacrificed one each at 12, 36 and 60 hours without tritium injection but with a sham saline injection.

The second control group for each Run (Group "C") constituted a control for the amount of incorporation of metabolite which would have occurred independent of tooth movement. It consisted of 6 animals sacrificed one each at 12 hour intervals. Two hours before sacrifice each animal received an intraperitoneal injection of an appropriate tritiated

metabolite. No elastics were placed.

In Run #1 each metabolite-injected animal received 1 micro-curie of H^3 - thymidine per gram of body weight. (After Dale & Hunt, 18.)

In Run #2 each metabolite-injected animal received 1.75 micro-curies of H^3 - proline per gram of body weight. (Based on a preliminary run by the author and D. L. Buck.)

In Run #3 each metabolite-injected animal received 6 micro-curies of H^3 - uridine per gram of body weight. (After Stallard, personal communication to D. L. Buck.)

The rationale for the choice of these metabolites is indicated in the Introduction and treated in greater detail in Appendix F.

Animals of all Runs and of each group within each Run were handled as similarly as possible, all being caged in the same room at constant temperature, and fed the same diet. Food and water were available ad libitum until four hours before sacrifice at which time the solid ration only was removed. In order to reduce the likelihood of loss of elastics, the solid ration (Purina Laboratory Chow) was ground and supplied as a powder. An attempt was made to control for diurnal variation (55) by pre-experimental acclimitization for one week in a continually lighted environment. The same lighted environment was maintained during the experimental period. Animals were weighed at specified intervals during the experimental duration to obtain relative data as to health and growth.

Note: The injectables employed were:

Thymidine methyl H^3, specific activity 14.1 micro-Curies/millimol (NET - 027),
L proline H^3, specific activity 5.0 micro-Curies/millimol (NET - 090), and
Uridine H^3, specific activity 8.25 micro-Curies/millimol (NET - 028).

All ~~was~~ were obtained from the New England Nuclear Corporation.

At the appropriate times the animals were sacrificed by decapitation. Each maxilla was immediately removed and fixed in 10% neutral buffered formalin. In addition to the dental tissues, a 2.5 cm length of the upper small intestine was obtained from each experimental animal, starting just distal to the pyloric end of the stomach. The rest of the carcass was preserved separately by freezing for possible later use. After fixation, the maxilla of each animal was divided in half in the mid-sagittal line and each half was decalcified in formic acid-sodium citrate solution, run through the usual dehydration procedures, and imbedded in paraffin. During the decalcification and imbedding procedures, tissues from all the animals of each Run were processed at the same time and in the same solutions.

After imbedding, each block was sectioned parasagittally. The sections were cut on an AO Spencer #815 Rotary Microtome at a thickness of 7 to 10 u. Most H^3 emissions, however, do not travel over 1 u in tissue. In tritium autoradiography consequently, sections thicker than 3 u are considered infinitely thick. In effect we measure metabolite incorporation in only the top 1 u of each tissue slice. (86, p. 295) Therefore, for practical purposes, we may say that we are counting the radioactive emissions from a surface or, to be more precise, from a standardized thin slice of tissue.

Criteria were established for satisfactory section

orientation in three planes of space. Antero-posteriorly, an attempt was made to have the section cut through the crowns of all three molar teeth to insure para-sagittalness. Vertical and buccolingual orientation were considered satisfactory when the pulpal soft tissue was observed to run continuously and without interruption from the pulp chamber down through the open apical portion of the root. (An oblique section would have gone through the buccal or lingual wall of the root yielding a continuous elipse of root structure at the apex. A section not centered buccolingually would have shown hard structure overlying portions of the pulp chamber or pulp canal.)

Sections were prepared autoradiographically by the dipping method (40, 45, 56) using NTB-3 emulsion (Kodak). Three non-contiguous sections, each separated from the others by a minimum of 14 u, were selected from among the slides from each half of each maxilla. Specimens from the treated and untreated sides of the same animal were processed simultaneously. After being dipped, slides were stored in darkness at 4° C. for two weeks. On the fourteenth day the slides were processed photographically. After development for one minute in Dektol and fixation for two minutes, the slides were stained lightly with Harris' hematoxlyn to facilitate histologic examination.

In order to facilitate replication of measurements, all data evaluation was done from photomicrographs. Using a

Zeiss Photomicroscope, two photographs were taken of each utilized section. One photograph represented the PDL of the "pressure" area at the alveolar crest on the mesial aspect of the mesial root of the first molar (Area "M" of Fig. 2, Appendix H). The other photograph was of the "tension" area on the distal surface of the same root just apical to the furcal curve (Area "D" of Fig. 2). Photographs were made with standardized techniques and standardized orientation of sections, but inasmuch as the furcal curve tended to vary considerably from section to section, obtaining analogous "tension" side photographs presented greater problems than was the case on the "pressure" side. All photomicrographs were taken on Panatomic X film (Kodak) and were processed under standardized conditions in D76:H20 (1 to 1) for seven minutes at 68° F. Following fixation, standardized 5 x 7 enlargments were made of the approximately 1,200 negatives. These enlargements constituted the major source of data evaluated in this experiment.

In each of the scoring procedures, all photographs were evaluated. None was rejected for any cause. Within Runs, all photographs were coded and randomized and all scoring procedures were blind.

Each of the enlargements was first overlaid with a sheet of translucent acetate and a tracing of the periodontal ligament area was made. These tracings were cut out and weighed. The true area of the microscope field was determined

by photographing a stage micrometer. Since the weight of acetate per unit of area was known, introduction of a simple conversion factor enabled calculation of the PDL area for each photograph.

Evaluations of metabolic activity were then made. The enlargements for the thymidine Run were again trans-illuminated and all labeled cells within the PDL area were counted. A labeled cell was defined as one with six or more silver grains over the nucleus. Counts for 30% of the thymidine photographs were replicated. All counts were recorded on acetate overlays so that the source material and primary data would remain unaltered and available for subsequent check.

In the cases of uridine and proline, the pattern of metabolic incorporation was general. Counting of labeled cells was not meaningful since almost all sectioned cells could be expected to show some label. Therefore the metabolic activity for each of these two metabolites was evaluated in the following fashion. Within each Run, the randomized photographs were examined individually by trans-illumination and distributed into seven categories ranging from least dense to densest in terms of autoradiographic counts in the periodontal ligament area. After the tally was complete, the photographs were re-randomized and re-tallied using the same procedure. Yet another replication was then made so that three independent subjective evaluations as to the density of each photograph were available. Reproducibility of ratings

was high. From among those photographs in each Run which had been consistently classified in the same category during each of the three evaluations, a sub-sample was selected consisting of seven photographs chosen at random from each of the seven density categories. This procedure was done separately for uridine and for proline. Each dot over the periodontal ligament area of each of these photographs was now counted and recorded upon an acetate overlay, using a Klett Colony Counter. Using the previously obtained PDL surface area values for each photograph, the number of counts per unit area was calculated for each of the photographs in the Run which had yielded similar subjective assessments. In this way relative density values for both proline and uridine activity were obtained which appear valid within Run but have no between-Run significance.

Several additional types of measurement were made. As an approximation of relative force residual in each elastic at the time of the termination of the experiment, the shortest distance between the enamel surfaces of the first and second molars was measured for each experimental slide using a 10X objective and a 10X ocular with an ocular micrometer. In the thymidine Run, the distance between the first and second molars on the control side was also determined.

An attempt was made to control for individual variation by introducing a corrective factor based on the relative uptake in the gut of the injected metabolite. A specimen was

taken from the duodenal area for each of the thymidine animals. This was dried, pulverized and evaluated using a scintillation spectrometer (Packard Tri-Carb). It was found that the values for gut uptake varied widely among the animals and it was felt that this variation was largely artifact due to the self-quenching effect of the gut samples, the problem being compounded by our inability to grind samples consistently. This treatment was thus considered unreliable and was not utilized in the uridine and proline runs.

CHAPTER V

RESULTS

Tests of Experimental Execution

Before discussing the experimental findings per se, it is appropriate to indicate certain tests of experimental execution which were performed in order to obtain some idea of the limits of reliability of the experimental model.

Determination of measurement error

Previous tooth movement experiments using the molars of rats have characteristically involved descriptions of contra-lateral differences in a small number of unilaterally treated animals. The assumption has been that differences discerned between sides were entirely attributable to the primary treatment. Implicit in this line of reasoning is the antecedent assumption that contra-lateral differences within untreated control animals are negligible or non-existent. This antecedent assumption was tested in the course of the present study using the control groups for each of the three Runs, in a fashion detailed in Appendix G.

From these tests it was concluded that there are indeed very significant contra-lateral differences within untreated animals. These differences are probably in part

true biological differences and in part the effect of technical errors of the model. In either event, in terms of the goals of the present experiment they constitute a form of measurement error sufficiently great so as to invalidate any interpretations based on individual selected slides. This finding confirmed us in our intention to avoid selective treatment of the material and to confine ourselves to recording only statistical and observational findings derived from the use of the total sample.

The existence of a sizable measurement error always renders it more difficult to obtain statistically significant findings even in the presence of a true difference. (The reasons for this statement are indicated in Appendix G.) Therefore the presence of a consequential measurement error does not operate against the type of findings reported herein. However, the fact of high contra-lateral variability should constitute a strong injunction against attempts to develop theoretical constructs based on extrapolations from findings on small numbers of selected slides.

Propriety of between-run comparisons

If valid comparisons are to be made between Runs, it is necessary to establish that the animals of the three Runs had similar characteristics and were similarly affected by the primary experimental treatment (elastic placement). It would also be desirable to establish that the three

injectables were of approximately equal toxicity and that they were not significantly more toxic than the sham saline injection given the background group of animals.

Three measurements of animal weight are available for checking the above relationships. The most valuable are GROWTH which measures weight gain from elastic placement to time of injection, and WTLOSS which measures the diminution in weight during the two hour period from metabolite injection to sacrifice. (Many weights would be expected to decline during this period even if the injection were completely benign, since this is also a period of solid food deprivation.) The third measure, WTINJ weight at injection, is of slightly less significance since the base line weight which it reflects is much less important as the rate of change. (Note: for descriptions of variables, see Appendix A, p. 1.)

Comparing the three control groups (N = 6 in each Run) we find that there are no significant differences among them in terms of any of the three values at the .10 level or better. (It must be noted that the failure to demonstrate differences does not demonstrate that they do not exist [error of accepting the null hypothesis].) However, the failure to find significant differences implies that such differences as may exist are probably not very great.

Comparing the experimental animals between groups (N = 24 for each Run) we find that there are no significant differences for WTLOSS or GROWTH. This permits the inference

that the animals in the three Runs grew similarly and were similarly affected by injection. However, in terms of WTINJ, we find that the thymidine animals were significantly larger than the proline animals at the .05 level (T = 2.359) and larger than the uridine animals at the .01 level (T = 2.750). The average weights of the animals in the three groups were 88.9 gms (thymidine), 81.5 gms (proline) and 81.1 gms (uridine); the sigma in each case being around 10 gms. It may be noted that the average rate growth for rats of this size and species is 40 grams per week. A difference of 8 grams therefore corresponds to an age difference of approximately two days.

Meaningful statistical comparisons for the difference in effect of the injected metabolites and the saline sham cannot be made since some data is missing and the N for each background group ("B") was only 3 at the outset. It is only possible to say that no gross differences in effect were found. (The background groups of animals were generally used only as a technique control and were dropped from the statistical analysis entirely.)

A further requirement of any statistical design is that the individuals chosen for study be randomly selected. A measure of our success in fulfilling this requirement is the fact that the correlation between animal weight at ligature placement and the number of hours to sacrifice is .017 for all animals used in the experiment. The close approximation

of this value to zero tends to indicate that the animals were chosen for the different time points without bias.

Findings

Having thus indicated the reasons why we consider our data to be appropriate measures of the parameters with which we are concerned, we may now examine the data themselves. These yield findings in three categories.

1. General observations,
2. Biological (metabolic) findings, and
3. Biomechanical (dimensional) findings.

The raw data has been collated and is attached as Appendix A which also defines the variables for which statistics have been accumulated.

General observations

Distribution of label.--It seems quite important to note that each of the three metabolites yielded considerable labeling in the PDL. In fact, if we exclude the expectedly consistent finding of considerable thymidine label in the basement layer of the gingival epithelium, the heaviest incorporation of each metabolite anywhere in the periodontal tissues was in the PDL. Such a finding strongly implies that metabolic turnover rates in the PDL of animals of the type studied are quite high and that we are dealing with a more metabolically active tissue than has been generally assumed. Heavy labeling on the PDL was found on both the treated and

untreated sides of the experimental animals as well as bilaterally in control animals.

Effects on the untreated side of control animals.--A second noteworthy finding with all three metabolites was the fact that unilateral placement of an elastic had contralateral effects. This finding, which supports previous observations by Stallard and Buck, but is contrary to those of Waldo (93, p. 484), may now be considered characteristic of the model at a high level of confidence. It is particularly evident on the distal ("pressure") side where with regard to each metabolite the condition following unilateral elastic placement yields bilaterally associated similar effects at the .05 level or better. (See Appendix B, Tables A through F.) As a matter of fact, correlations between similar areas on the treated and untreated sides of experimental animals tend to be higher than between the two untreated sides of the control animals. (Compare Appendix B, Tables A through F with corresponding Tables A' to F'.)

This finding becomes particularly interesting when we consider that the dentitions of rodents are not characterized by continuous tooth contact but rather present large edentulous gaps mesial to the first molar. Thus we cannot ascribe the effect on the untreated side to alterations in contact point pressure around the arch.

The significance of this effect is such as to bring into focus a limitation on the conclusions which may be drawn from in-animal controls using the present experimental model. Certainly it appears that parallel studies of completely untreated control animals are required to establish a base line.

Diurnal effects.--Diurnal effects in metabolic rate which may have occurred during the course of this experiment were measured using the variable TIMSAC and were not found to correlate significantly with any other variable. It seems reasonable to conclude that such diurnal effects as do exist are either not large or are non-linear.

Pre-treatment variations in PDL width on opposite sides of the root.--It has been assumed that the root of the untreated tooth would be found centered in its socket. Thus we expected to find the photographic fields on the mesial and distal sides of a given root approximately equal in area. This did not prove to be the case. (See Appendix B, Table 7, lines 7 and 11) In untreated animals, the average area on the distal side (which in treated animals became the "tension" side) was 40% greater than that on the mesial side (which in treated animals became the "pressure" side). This finding represents an area difference significant at the .01 level ($T = 3.781$, $N = 17$). Its effect is to preclude direct comparisons of dimensional or metabolic activity changes

between the two sides of the root unless suitable adjustments for the pre-experimental differences in dimension have been made. To our knowledge analogous adjustments have not been made in previous work with the present experimental model.

Biologic (metabolic) findings

The specific biologic findings are summarized in columns 1 through 6 of Table 5-1 which abstracts the data detailed in Appendix B, tables 1 through 6 inclusive.

The processes of analysis by which the tabulated results were derived from the raw data are described below together with a brief consideration of the meaning of the findings. Each metabolite is considered separately.

Thymidine incorporation in the PDL.--Examination of the data from Run #1 revealed considerable variation in the rate of new cell formation between animals similarly treated. Analysis of variance through time (trend analysis) revealed that because of the magnitude of this variation, curves connecting time point means did not yield statistically significant results. The problem was not that curves connecting the average values for the pairs of animals at each of the twelve time points could not be drawn. It was rather that the disparities between the values for the pair of animals at each time point tended to be sufficiently great that averages between them were not meaningful. The number of animals at each time point was too small to allow the

construction of significant rate versus time curves.

All experimental animals were thenceforth treated as a single group and tested against the group of all control animals. With respect to total replicating cells in the PDL per photographic field (CC variables), and also to replicating cells per unit of PDL area (CA variables), the two groups were T-tested against each other. Following this procedure, correlated means T-tests were performed testing the mesial and distal PDL areas on the treated side of experimental animals against their in-animal untreated contra-lateral equivalents. As a further control, similar correlated means T-tests were performed within the control group to be sure the two sides of the untreated control animals did not differ significantly from each other.

The results of these tests are shown in Appendix B, Table 1 (total cells/PDL field) and Table 2 (Cells/100,000 square u) and in columns 1 and 2 of Table 5-1.

They may be summarized as follows:

On the "pressure" side the average cell replication rate in the experimental group is higher than in the control group. However, because of the considerable variation in replication rate within the sample, this difference falls short of statistical significance at the .10 level ($T = 1.347$), ($T.10 = 1.701$). Nonetheless, we may tenably state that, given the observed values, the probability is less than .10 that the rate of cell replication in the control animals

exceeds that in the experimental animals, as would be required by conventional hypotheses. (In a one tailed T-test, the .10 level is T = 1.313 with df = 28.) We may further state that the cell replicational activity is significantly higher on the treated "pressure" side than on the untreated contralateral side. (α = .05, Appendix B, Table 1, α = .10, Table 2.) This finding clearly contradicts the hypothesis that total cell replicational activity drops on the pressure side. The further consequences of this finding will be explored in the discussion.

On the "tension" side the conventionally predicted increase in cell replicational activity did occur (α = .10). In this case there were no statistically significant differences between treated and untreated sides within the experimental animals, but there was a tendency in the direction of higher activity on the treated side as is conventionally stated to be the case.

Conventional hypotheses predict opposite directions of change in cell replication rate on the tension and pressure sides of treated teeth. The consequence of such biologic activity would be expected to be a high negative correlation in cell replicational activity on the treated "pressure" (right mesial) and "tension" (right distal) sides of experimental animals. This was not found to be the case, and in effect the correlation between the two was mildly positive (r = .220, not significant).

No significant differences in metabolic activity rate on the "pressure" and "tension" sides were found once allowance was made for area differences.

Proline incorporation in the PDL.--As with thymidine, the variations among the proline values were sufficiently great to disallow the construction of meaningful time-rate curves. A set of T-tests was therefore performed similar to those for Run 1. The findings are indicated in Tables 3 and 4 and in Table 5-1, columns 3 and 4. They may be summarized as follows. On the "pressure" side, proline incorporation falls as was predicted. However, on the "tension" side, where increased incorporation would have been predicted, we find that incorporation also falls. Similar reductions occur on the untreated side of experimental animals. These findings occur at high significance levels and constitute grounds for believing that the initial response to tooth movement in these animals involved a generalized reduction in collagen synthesis in the PDL. How general was this reduction is indicated further by the highly significant positive correlations in Tables C and D where significant negative correlations would again have been predicted.

Within the experimental animals, the activity on the "pressure" side is significantly lower than that on the "tension" side even when corrections are made for area differences ($a = .01$). On the untreated side of experimental animals the same relationship exists between the "pressure control" side

and the "tension control" side. The consequences of these findings will be considered in the discussion.

Uridine incorporation in the PDL.--Data from Run #3 was handled in a manner similar to the others. T-test findings are shown in Tables 5 and 6 and in Table 5-1, columns 5 and 6. It will be noted that there was a consistent pattern of increase of uridine incorporation on both "pressure" and "tension" sides incident to tooth movement ("pressure" side $\alpha = .05$, "tension" side $\alpha = .01$). As a first estimate, we may therefore infer that RNA synthesis and therefore total metabolic activity levels as an initial consequence of inducing tooth movement. Little or no contra-lateral in-animal effect was measurable on the untreated side of experimental animals. However, there was a significantly higher activity level on the "tension" side than is found on the pressure side of the same treated animals even after corrections for area differences are made.

It should be stressed that uridine incorporation is a measure of total bio-synthetic activity and not of the specific bio-synthesis of collagen or any other single protein. Much of any increase in uridine incorporation could easily go to the production of catabolic enzymes. Furthermore, the fact that the difference in uridine incorporation rate between the two untreated sides in the control animal approaches statistical significance must unfortunately cause us to view these findings with reservations. This will be

considered further in the discussion.

Bio-mechanical findings

The bio-mechanical (dimensional) findings in the PDL are summarized in the last column of Table 5-1 which abstracts the data detailed in Appendix B, Table 7. First it is appropriate to indicate the magnitude and nature of the dimensional changes at the site of the primary treatment--the location of the elastic itself. The space between the first and second molars which appeared following elastic placement was dramatic and consistent. The extreme range limits of the treated and untreated sides did not overlap. (T-ratio = 16.7 with df = 22.) Measurement of the treated side space revealed a mean separation of 516u and a sigma of 63u. Measurement of the space on the untreated side yielded a mean of 160u and a sigma of 31u. Contrary to the experience of Waldo and Rothblatt, and Yen and Rothblatt, but similarly to that of Macapanpan, the separation of the teeth did not appear to occur gradually throughout the experimental period. There appear to be no significant differences in separation between the six hour and the 72 hour animals. It is evident that the major changes induced by the elastic occurred quite quickly sometime during the earliest hours, yielding a relatively mild residual force during the remaining experimental period. In this sense the force acted more like a classical intermittent force than had previously been suspected.

On the other hand, the residual force was certainly not light. An attempt to quantitate it was made by testing elastics of the type used on an Instron materials tester with a specially prepared jig. The results of this test and calculations from it appear in Appendix E. On the basis of our findings we may reasonably conclude that the original total force originally approximated 50 - 75 grams but rapidly fell to 2 to 4 grams over a period of less than 6 hours. This latter force was then maintained fairly unaltered for the duration of the experiment. Estimating the root surface area very liberally, we may conclude that the terminal pressure was on the order of 60 to 80 grams per square centimeter (see Appendix E). In general, therefore, while the initial force was no doubt extreme, the pressure exerted during the major portion of the experimental period, though heavy, was probably less than has been previously assumed for this model. It is probable that pressures of this order are at times developed clinically with brass separating wire, with rectangular wire closing loops, or when torquing individual teeth.

Turning to the PDL itself, we were quite surprised when measurements revealed that, notwithstanding the enormous separation produced at the primary site of elastic placement, the dimensional changes in PDL width on both the "pressure" and "tension" sides were exceedingly small. Table 7 reveals that the reduction in periodontal width on the "pressure" side

is not significant when compared to corresponding areas in untreated control animals. (T-ratio + -.9326 df = 88.) A reduction in the "pressure" side width is found at the .05 level when compared to the contra-lateral in-animal untreated side, but the average reduction in PDL width is only 10%. (It should however be noted that the "pressure" side width is positively correlated significant at .01 level, r = +.356, N = 72 with the length of time from elastic placement to sacrifice. This finding implies that there may be greater reduction in pressure side width early in the experimental period with later recovery. The correlation of experimental duration (TIME) with the dimensions of the "tension" side and control PDL areas approaches zero.)

The possibility that the opening of space between the first and second molars is accommodated for by approximation of the apices of their adjoining roots may be ruled out since measures of the distance between these apices on treated sides are slightly, though not significantly, larger than those on the untreated sides (see Table H, Appendix B). We can be quite sure the true distance is not reduced and we are confronted with the fact that considerable translation of crown position has occurred not accompanied by major compression of periodontal ligament on the "pressure" side at the point at which maximal compression was to have been expected. This physical finding can be explained only in terms of dimensional changes within the alveolar bone itself. The consequence of this finding will be considered in the discussion.

CHAPTER VI

DISCUSSION

It is now appropriate to consider the degree to which our findings tend to confirm the hypotheses on tooth movement which were outlined in the Introduction. Let us first consider the question of cell replicational activity. Here the conventional expectation had been that replication rate would increase on the "tension" side and decrease on the "pressure" side while showing little or no change on the untreated contra-lateral in-animal control sides. The results of the experiment confirm our expectation on the "tension" side, where the expected increase in activity is found reliable at the .10 level. (See line 6, Tables 1 and 2.) Also corroborating our expectations, comparatively little change is found on the untreated "pressure" control side. However, whereas little or no change in activity level had been anticipated on the untreated "tension" control side, we find an increase in rate of new cell formation significant at the .10 level. In point of fact, the rate of new cell formation on the untreated "tension" control side approaches that on the treated "tension" side and is markedly greater than that of comparable sides in untreated control animals as shown below.

New Cells Per PDL Field (from Table 2)

	$\bar{x}$	α
"tension" side	9.33	10.00
in-animal "tension" control side	6.98	6.36
control animal analog	2.65	1.70

Of even greater biologic interest is the finding of a tendency toward increased cell replication on the experimental "pressure" side. The comparison of cell formation on the experimental "pressure" sides with comparable areas in control animals follows below.

New Cells per PDL Field (from Table 2)

	$\bar{x}$	α
"pressure" side	6.78	5.96
in-animal "pressure" control side	4.25	3.11
control animal analog	3.36	3.16

Because of the high variability in the experimental sample, one may not properly say that the treated "pressure" areas are significantly more active than the analogous areas in the control animals. But we may say that the probability that the conventional hypothesis is correct, and that cell replication is significantly reduced on the "pressure" side, is considerably less than .10. And the patterning of results does indeed give reason to believe that the differences recorded are true differences. The differences in means between

experimental and control animals are quite large, amounting to doubling on the "pressure" side and trebling on the "tension" side. Conclusions at higher levels of significance are not forthcoming because the t-test penalizes heavily for the very consequential variance. A major contribution to the high variance in the experimental group resulted from the pooling of animals from different time points. The specific question as to whether cell replicational activity on the "pressure" side actually does increase could probably be definitively answered by replication of the present study with all experimental animals grouped at a single time point in the mid-range between 6 and 72 hours. In such an experiment the experimental and control groups should best approximate each other in size.

It is perhaps noteworthy that ours is not the first experimental observation of increased cellular activity on the "pressure" side. Reitan, for example, reports a similar observation in humans. He ascribes the phenomenon to the fact that his subjects were young "so that the supporting tissues already contained a maximum of connective tissue cells previous to the experiment." This explanation seems tenuous, however, since his control individuals were presumably no older than his experimental ones. It is not

appropriate to contend that the picture in older animals would show the same trend we see in the present study. That can be determined only by experimental work with older animals. But it is appropriate to say that "pressure" side changes in our own experiment and in Reitan's indicate that the conventional hypothesis requires re-examination.

With regard next to proline incorporation, our hypotheses would have led us to expect increased activity on the "tension" side with decreased activity on the "pressure" side; the untreated contra-lateral side to have remained relatively unaffected. These expectations were not satisfied. Rather we find a consistent and significant drop in all tested areas in experimental animals. This finding is demonstrated in Tables 3 and 4 and again in Tables C and D. The last two tables are perhaps the more interesting since they demonstrate quite conclusively the generalized positively correlated changes induced by the elastic. Since proline incorporation is generally accepted as a proper measure of collagen synthesis (see Appendix F), we may maintain that the treatment of one area with an elastic produced a decline in collagen synthesis in all of the examined regions. It seems appropriate to ask why collagen synthesis appears to decline rather than rise as an initial response to tooth movement. This is likely related to the fact that base line turnover rates of collagen in living tissues are quite considerable--a phenomenon which investigators did not surmise

before the availability of radio-tracers. The work of Bentley and Jackson on whole body collagen turnover, and the work of Claycomb with particular reference to gingival collagen turnover indicate that the material is not nearly so stable and immutable as had been supposed by Oppenheim, Sicher, et al. And the recent work of Carniero indicates that collagen exchange at the alveolar crest in young rats is higher than in any other PDL area with the possible exception of the apex (14) Perhaps the placement of the elastic brings about a transient generalized lowering of the high ambient rates of fiber formation. It seems reasonable that such a diminuation in collagen production would be recouped at some later point in the process of physiologic accommodation to the exogeneous force. Confirmation of this assumption would require an experimental model which could be followed beyond the initial phase of tooth movement.

It is appropriate to note that our results in determining rate of proline incorporation, while new in the orthodontic literature, parallel the findings of Crumley using a similar model as reported in the periodontic literature. (17)

In predicting the results of the uridine run, fewer guidelines were available since monitoring of this measure of metabolic activity has not previously been attempted in the PDL. In general, had conventional tooth movement hypotheses held, we would have expected no differences in activity rate on the untreated side of experimental animals

as compared to untreated controls. On the treated "tension" side, we would have anticipated increased RNA synthesis as a necessary precondition for the expected increase in collagen synthesis. However, this anticipated increase in collagen synthesis did not occur, and, in fact, the opposite was the case as we have seen above. On the treated "pressure" side, some degenerative changes were anticipated, such as disorganization of fibers. However, these degenerative changes might easily have been accompanied by an increase in RNA synthesis, if they did in fact occur. This is true because even degenerative changes in living organisms (other than necrosis) are active and orderly processes. They therefore increase output of catabolic enzymes in whose manufacture RNA is utilized.

In the event our experiment recorded statistically significant increases in RNA synthesis on both the treated "pressure" and "tension" sides of experimental animals, as compared to corresponding areas in control animals. (See Table 5-1.) No significant differences appeared between untreated sides of experimental animals and similar cases in control animals. Also, no significant differences were demonstrated between analogous areas on the treated and untreated sides within the experimental group. However, the variation between sides in the control animals was quite large and in fact approached statistical significance. This finding dictates that extreme caution be used in interpreting

the results of this Run and severely limits the confidence we may have in any conclusions drawn from it.

Dimensional Changes

An understanding of the experimental findings concerning the location and magnitude of dimensional changes incident to elastic placement is obtainable by referring to Figure 3, Appendix H. The placement of an elastic at "A" produced an average increase in the distance between the teeth in that region to 335u. (Experimental RLIGW minus Control LLIGW equals effect of treatment.) Under the same treatment, the distance between the apices of the approximating roots of the two teeth ("D") showed no change (see Table H, Appendix B). We may say therefore, that we are dealing with a practically pure tipping movement whose axis is located at or near the distal root of the first molar.

It is reasonable to assume that the crown of the first molar tipped mesially no less than 200 u while the rest of the space at "A" accrued from the distal displacement of the second and third molars. The average pre-treatment width of the PDL at "B"--the "pressure" side--was 90 u. Thus during the course of an experimental period far too brief for any significant biological adjustment within the PDL to have occurred, the first molar moved mesially twice the total width of the PDL. During this period the average reduction in PDL width on the "pressure" side "B", approximated 9 u.

The corresponding average increase in width on the "tension" side "C" was even smaller.

Thus we are confronted by the fact that a force sufficiently strong to open a space of 18 mils between two teeth in a 6-hour period produced an average reduction of width on the "pressure" side of the PDL less than twice the diameter of a red blood corpuscle with no measurable widening at all on the "tension" side.

This remarkable resistance to dimensional change appears surprising in terms of conventional representations of the PDL as having a ground substance from which water is easily expressed under load. These, however, involve an archaic concept of the nature of connective tissue in general (9, p. 20) and of the PDL in particular (see Appendix C). In a word, the PDL appears to act like a rubbery structure--not a watery one.

The failure of the PDL to alter dimensionally in the predicted manner emphasizes the propriety of re-examining the entire pressure-tension hypothesis. To Schwarz and Reitan, it seemed obvious that force application caused tooth movement within the periodontal space yielding an increase of pressure on one side of the root and a reduction of pressure on the other. (76) The essence of the "pressure-tension" hypothesis is the assumed influences of assumed differences in pressure level on opposite sides of the root. But if the PDL is a hydraulic system, as has been clasically maintained,

such differences are impossible even without the evidence of the findings outlined above. This is the case because if the PDL is a confined fluid the ambient field pressure on the "tension" side will be precisely the same as that on the "pressure" side after only the briefest of inertial lags at initial force application. That is the precise consequence of the action of Pascal's Law. Therefore, any tissue changes which are a function of alteration in ambient field pressure within the PDL will be reflected equally on both the "pressure" and "tension" sides of the root. We feel that it is appropriate that the profession reconsider the ~~extra~~ entire "pressure-"tension" hypothesis with a view towards abandoning it or modifying it greatly.

There remains the possibility of mechanical alterations to the solid components of the PDL. These might include crushing of fibers and blood vessel walls on the "pressure" side, and distension of fibers on the "tension" side. The present experiment by its nature, can shed no direct light on these questions.

The above findings of dimensional change are utterly incompatible with the idea that the tooth moves only within the periodontal space and that the bony walls of the alveolus are unchanged in position by the effects of the tooth-moving force. Ample evidence has been adduced to prove that, within our experimental model at least, the bone does indeed bend. Nor should this finding really surprise us as clinicians.

Every practicing orthodontist has had occasion to observe immediate movement greater than can be explained by changes confined within the limits of the PDL. This phenomenon occurs most frequently when tying in isolated teeth out of line; particularly incisors where the overlying cortical plate is relatively thin. Similar immediate gross changes in tooth position or inclination may be noted quite generally when torquing forces are employed. Perhaps as clinicians we do not wish to see such gross movement, but it does occur, most often with no observable deleterious sequelae. In prosthetic dentistry, incidentally, it has been fairly well established that extreme openings of the mouth, volitionally and within physiologic limits, routinely causes measurable flexion of the body of the mandible. (52) It is interesting to speculate that the slow process of en masse movements as compared to individual tooth movements may occur because in the latter procedure the clinician unknowingly produces flexion in the bone.

The attitudinal set against admitting even the possibility of bone bending as a consequence of force application is well typified by Gottlieb's statement that "contact between tooth and bone determines the <u>limit</u> of the tooth movement. No matter how much you increase the force, a further movement of the tooth is <u>impossible</u>. If we use ligatures, we will rupture them trying to achieve further movement. The rupture of a tightened ligature seems to be a sign of existing

contact between tooth and bone. . . . We have found that the natural limitation of tooth movement is contact between tooth and bone. No technique can eliminate that possibility. . . . No force can achieve more than contact between tooth and bone, and no technique can avoid it." (29) (Gottlieb's emphasis.)

And to show how strongly this attitude persists, we may note that Bien analyzes the results of his recent experiment as if the entire force expenditure occurs within the PDL space even after reporting that in one case in sixteen the loaded tooth was intruded entirely through the maxilla. (6) Certainly the PDL cannot be that wide!

CHAPTER VII

SUMMARY AND CONCLUSIONS

1. Contrary to expectations from classical hypotheses, no qualitative differences in metabolic response were noted between "pressure" and "tension" sides of experimental animals. In terms of cell proliferation, both "pressure" and "tension" sides showed tendency to rate increase when compared to corresponding areas in untreated control animals. In terms of collagen synthesis, both "pressure" and "tension" sides showed statistically significant decreases in rate of activity.

2. In both the Proline and Thymidine Runs, there were evidences of significant metabolic effects on the untreated sides of experimental animals. Wherever such changes were found they were in the same direction as those on the treated side but of reduced magnitude.

3. Results of the Uridine Run were inconclusive and cannot be considered more than a first approximation of changes in the general rate of cell metabolism.

4. Measurement of the periodontal ligament in untreated controls revealed that the mesial root of the maxillary first molar was not centered in its socket. The periodontal ligament on the distal side was markedly wider than

that on the mesial side in the region studied. Since the "pressure" side is the already narrower mesial side in our model, caution must be exercised in ascribing "compression" to the experimental treatment.

5. Introduction of the elastic between the teeth produced an initial force approximating 40 grams which fell off rapidly within the first few hours of the experiment. By six hours the residual force had fallen to 5 - 7 grams at which value it remained until sacrifice. These forces produced an initial pressure of approximately four kilograms per square centimeter and a final pressure approximating .6 kilograms per square centimeter. Notwithstanding these very high pressures, the width of the ligament on the "pressure" side decreased an average of 9 u or less than 10%, while the width on the "tension" side was unchanged.

6. Introduction of the elastic increased the separation between the crowns of the first and second molars an average of 335 micra but produced an average change of less than 15 micra in the distance between their approximating roots. We conclude, therefore, that the first molar was tipped with an axis of rotation near or at its mesial root.

7. Since the crown of the first molar was displaced markedly with only negligible change in the width of the PDL, we conclude that the movement involved deflection of the alveolar bone. The fact that a force capable of bending the bone produced only minor dimensional changes within the PDL

implies that that structure has a resilient or rubbery consistency rather than the watery character ascribed to it by classical histology. This finding is consistent with modern biochemical studies on the nature of connective tissue ground substance (see Appendix C).

8. The general utility of a standard model for tooth movement experiments has been assessed. It appeared that, while this model is capable of yielding considerable interesting information, the variability within and between animals is sufficiently great so as to render suspect any rate curves plotting metabolic activity against time unless considerably larger numbers of animals are used at each time point than has thus far been attempted.

9. It seems appropriate to offer for further testing some altered hypotheses on the nature of the metabolic and dimensional changes which occur in the periodontal structures when teeth are moved.

It is proposed that the delivery to a tooth of either a continuous or an intermittent force within the limits of physiologic tolerance yields two types of change. The first type involves the periodontal ligament and the lining of the bony alveolus. This is the phenomenon of resorption and deposition investigated so brilliantly by Oppenheim, Sandstedt and others. Some of the biological parameters of this type of change have been investigated in the present study and have been reported above. In general our findings

indicate that biological changes in this region are accompanied by smaller dimensional changes than has been thought. Also such changes in biological activity as do occur tend to have effects in untreated areas as well as in the areas directly subject to the tooth moving force. It must further be noted that rates of cellular activity and of fiber production were not found to be negatively correlated between "pressure" and "tension" sides as had been previously proposed.

The second type of change occurs through deflection of the entire dental unit. It was reported long ago by Farrar and Kingsley but has been studied only slightly in this century. Such a deflection need not be massive to be biologically significant. The present study indicates that deflection can be produced by forces of smaller magnitude than had previously been thought necessary, particularly in areas where the investing bone is relatively thin. Certainly deflection of bone can be produced by forces less powerful than those required to produce major dimensional change or necrosis within the PDL.

The physiological sequelae to such bone flexion might be as follows. Strains would be set up within the structure of the bone, as in any solid material stressed within its elastic limit. Renewal of the osteones of the Haversian systems as part of the normal turnover process would now occur under altered conditions. Perhaps the strains on the

apatite crystals of the inorganic fraction of the bone would organize and direct the pattern of bone renewal by producing electric effects as has recently been reported by Basset (4) and Picton (62). In any event, the new osteones would be laid down in accordance with Wolff's law in such a way as best to resist the stresses upon the system. Upon completion of the turnover process, if the exogeneous force was acting through a short enough distance, the tooth would again be at equilibrium within the dental unit. However, the bone spicules would now be arranged in a force-resisting pattern rather than a rest pattern, which might contribute to the relapse potential of the system unless it were retained throughout another bone turnover while in an unstressed equilibrium state.

It is to be emphasized that while the metabolic dimensional findings of this project are advanced at comparatively high confidence levels, these concluding hypotheses are not proven by any means, but are proposed simply as a basis for further investigation. However, a fairly rigorous attempt has been made to express them in what is, given present research techniques, a testable form.

VIII

APPENDICES

APPENDIX A

EXPLANATION OF VARIABLES AND RAW DATA PRINTOUT

Data has been accumulated from a total of 99 animals. The experimental procedures were sequenced in 3 Runs of 33 animals each. Data are available on slightly different variables for each of the three Runs. These variables are described below. The variable names are those used in data processing procedures and correspond to the column headings in the raw data printout which follows.

1. For each of the 33 animals in Run 1 thymidine, the following primary data were accumulated.

TIME. Time (in hours) from elastic placement to sacrifice.

WTINJ. Weight (in grams) at the time of metabolite injection.

WTSAC. Weight (in grams) at sacrifice.

WTANA. Weight (in grams) at anesthesia and/or elastic band placement.

GROWTH. Gain in weight (in grams) between elastic and metabolite injection (WTINJ minus WTANA).

WTLOSS. Loss in weight (in grams) between metabolite injection and sacrifice (WTINJ minus WTSAC). (This effect is partly the result of food deprivation during this period and

partly, perhaps, a result of the insult of the injection itself.)

GTBETA. A measure of the beta emissions per unit of weight per unit of time from a duodenal sample. These are comparative values among the experimental animals, the absolute values being of no importance for our purpose.

RLIGW. Distance in u between the first and second molars on the right (treated) side. (This value is a composite of three ratings, one from each of three sides.)

*Note: The right side, on which the elastic band is placed, will henceforth be termed the "treated" side. The left side, on which no elastic band is placed, will be termed the "untreated" side.

LLIGW. Similar to RLIGW above, but for the untreated side.

RCCM. The labeled cell count on the treated "pressure" side. (This value is a composite of three ratings, one from each of three slides, and of replications of 3/10 of these ratings.)

RCCD. Similar to RCCM above, but for the experimental "tension" side.

LCCM. Similar to RCCM above, but for the untreated "pressure control" side.

LCCD. Similar to RCCM above, but for the untreated "tension control" side.

RCWM. Area in square u of the PDL on the treated

"pressure" side. (This value is a composite of three ratings, one for each slide, and of replications of 3/10 of these ratings.)

RCWD. Similar to RCWM above, but for the treated "tension" side.

LCWM. Similar to RCWM above, but for the untreated "pressure control" side.

LCWD. Similar to RCWM above, but for the untreated "tension control" side.

For the next group of variables, each of the cell count values above was divided by the corresponding area value for the same animal yielding a cells per unit area value which was termed CA. Thus:

RCAM = RCCM divided by RCWM

RCAD = RCCD divided by RCWD

LCAM = LCCM divided by LCWM

LCAD = LCCD divided by LCWD

RAPEX = distance in micra between the apices of the distal root of the upper right first molar and the mesial root of the upper right second molar.

LAPEX = distance in micra between the apices of the distal root of the upper left first molar and the mesial root of the upper left second molar.

TIMSAC. The time of day at which the animal was sacrificed in hours and hundredths of hours. (Intended as control for possible diurnal effect.)

2. For each of the 33 animals in Run 2 (proline), the following data were accumulated.

TIME. Similar to Run 1.

WTINJ. Similar to Run 1.

WTSAC. Similar to Run 1.

WTANA. Similar to Run 1.

GROWTH. Similar to Run 1.

WTLOSS. Similar to Run 1.

RLIGW. Similar to Run 1, but available only for the 24 animals in which elastics have actually been placed. (LLIGW values on "contra-lateral" "control" side are not available.)

RDRM. The comparative labeling density in the periodontal ligament area on the experimental "pressure" side. This value is a composite of nine subjective ratings, three for each of three slides. (This value is a statement of comparative field density and is an estimate of the number of counts per 100^2 sq. u.

RDRD. Similar to RDRM above, but for contra-lateral "tension control" side.

LDRM. Similar to RDRM above, but for contra-lateral "pressure control" side.

LDRD. Similar to RDRD above, but for contra-lateral "tension control" side.

For the next group of variables each of the comparative labeling density values above was multiplied by the

corresponding area value for the same animal yielding an estimate of total grains per PDL field. This new group of variables was designated DOT.

Thus:

RDOTM = RDRM times RCWM

RDOTD = RDRD times RCWD

LDOTM = LDRM times LCWM

LDOTD = LDRD times LCWD

RAPEX = Similar to Run 1.

LAPEX = Similar to Run 1.

TINSAC = Similar to Run 1.

3. For each of the 33 animals in Run 3 (uridine), data are available similar to those for Run 2.

APPENDIX #A COMPOSITED RAW DATA THYMIDINE PAGE #1

	ANIMAL NAME	TIME	WT INJ	WTSAC	WTANA	GROWTH	WTLOSS	GTBETA	RLIGW	LLIGW
1	B1294	24.	86.8	79.6 ~~97.6~~	82.0	4.8	-7.2	92.652	191.333	205.333
2	B1107	66.	-0.0	84.3	70.0	-0.0	-0.0	91.167	448.000	126.000
3	B1211	72.	97.6	102.4	86.0	11.6	4.8	129.279	401.333	154.000
4	C1302	12.	82.8	81.2	74.0	8.8	-1.6	819.231	210.000	112.000
5	C1819	24.	91.5	90.8	86.0	5.5	-0.7	535.671	149.333	116.667
6	C1462	36.	66.9	66.0	63.7	3.2	-0.9	2312.333	168.000	121.333
7	C1835	48.	108.5	107.0	90.0	18.5	-1.5	491.533	168.000	158.667
8	C1367	60.	101.5	97.6	88.0	13.5	-3.9	3155.369	116.667	98.000
9	C1423	72.	78.5	77.5	65.5	13.0	-1.0	1490.710	149.333	135.333
10	X1520	6.	95.3	94.1	96.5	-1.2	-1.2	1519.106	443.333	154.000
11	X1757	6.	83.6	83.1	84.0	-0.4	-0.5	793.333	406.000	163.333
12	X1068	12.	83.6	81.6	84.0	-0.4	-2.0	715.915	485.333	140.000
13	X1985	12.	95.7	94.6	95.4	0.3	-1.1	725.407	480.667	238.000
14	X1466	18.	80.2	80.0	83.5	-3.3	-0.2	886.016	401.333	70.000
15	X1625	18.	82.0	81.8	78.5	3.5	-0.2	1773.950	583.333	107.333
16	X1040	24.	73.1	71.8	70.0	3.1	-1.3	501.572	606.667	177.333
17	X1958	24.	75.9	77.2	74.0	1.9	1.3	769.020	620.667	247.333
18	X1160	30.	94.4	92.0	88.7	5.7	-2.4	2133.437	476.000	177.333
19	X1343	30.	69.2	66.8	67.2	2.0	-2.4	1730.120	485.333	98.000
20	X1083	36.	93.6	91.4	89.6	4.0	-2.2	1142.235	340.667	121.333
21	X1424	36.	107.7	107.5	107.0	0.7	-0.2	2648.511	406.000	525.000
22	X1978	42.	75.8	71.9	65.2	10.6	-3.9	1009.441	532.000	140.000
23	X1095	42.	91.7	87.9	82.5	9.2	-3.8	2192.469	490.000	177.333
24	X1659	48.	107.2	103.6	96.4	10.8	-3.6	858.356	518.000	112.000
25	X1679	48.	86.2	85.1	85.0	1.2	-1.1	1753.469	494.667	224.000
26	X1078	54.	94.7	94.4	92.5	2.2	-0.3	2532.932	518.000	350.000
27	X1773	54.	89.6	89.0	80.0	9.6	-0.6	1498.058	462.000	191.333
28	X1290	60.	94.2	92.2	82.5	11.7	-2.0	1234.884	597.333	-0.000
29	X1065	60.	95.9	95.1	87.0	8.9	-0.8	1013.312	541.333	329.000
30	X1956	66.	88.3	77.2	74.0	14.3	-11.1	1713.920	480.667	200.667
31	X1794	66.	79.5	94.6	81.0	-1.5	15.1	1029.358	485.333	154.000
32	X1739	72.	91.6	92.7	78.5	13.1	1.1	2269.869	597.333	126.000
33	X1799	72.	103.7	108.1	86.0	17.7	4.4	1069.124	476.000	224.000

APPENDIX #A COMPOSITED RAW DATA PRINTOUT THYMIDINE PAGE # 2

ANIMAL NAME	RCCM	RCCD	LCCM	LCCD	RCWM	RCWD	LCWM	LCWD
1. B1294	0.000	0.000	0.000	0.000	3.126	3.943	2.258	3.366
2 B1107	0.000	0.250	0.000	0.000	2.506	1.970	2.539	2.493
3 B1211	2.250	1.250	1.500	1.750	3.089	3.026	2.520	4.309
4 C1302	7.500	3.750	7.500	6.000	2.571	3.548	3.145	3.613
5 C1819	0.667	0.667	0.000	0.400	1.647	3.774	1.556	3.892
6 C1462	0.000	0.750	2.000	0.000	1.910	2.586	3.245	3.524
7 C1835	1.750	5.000	9.750	1.500	2.263	3.936	2.445	2.337
8 C1367	3.500	3.000	5.000	2.750	3.416	2.752	2.390	3.505
9 C1423	6.750	2.750	1.500	2.250	2.304	4,098	2.721	3.361
10 X1520	1.250	1.000	3.500	4.000	3.143	4.834	2.984	3.388
11 X1757	0.250	4.250	0.750	0.500	2.861	4.609	4.155	3.118
12 X1068	3.667	4.000	7.000	5.600	3.008	3.508	3.256	3.581
13 X1985	9.200	7.400	8.250	4.750	2.342	3.070	2.711	4.724
14 X1466	1.250	0.500	1.250	6.500	1,642	4.303	3.286	3.553
15 X1625	0.750	3.500	11.250	19.250	1.963	2.349	5.405	5.313
16 X1040	8.000	44.750	5.000	26.000	2.808	5.740	2.675	3.919
17 X1958	1.500	11.750	3.333	10.667	1.702	3.724	1.901	4.761
18 X1160	9.000	12.333	2.200	9.000	2.823	4.483	2.499	3.432
19 X1343	6.667	6.333	2.400	10.200	2.939	4.254	2.529	2.903
20 X1083	4.333	10.000	5.400	17.200	2.231	4.101	1.955	3.956
21 X1424	19.000	1.000	4.250	1.750	2.076	2.238	1.920	5.261
22 X1978	3.600	13.600	2.333	5.667	2.335	2.992	2.900	3.470
23 X1095	8.000	10.000	11.333	7.000	1.810	4.105	3.386	3.543
24 X1659	23.000	17.667	7.000	2.500	2.815	3.002	2.240	2.388
25 X1679	4.000	5.500	1.750	6.250	3.390	3.567	2.022	3.156
26 X1078	4.667	26.667	1.400	12.200	2.918	4.589	2.984	4.529
27 X1773	8.667	17.667	4.800	3.200	2.406	2.806	2.634	3.912
28 X1290	0.250	0.000	0.000	0.000	2.776	1.938	2.592	3.598
29 X1065	4.250	1.500	6.000	1.667	2.543	3.614	3.851	3.441
30 X1956	11.250	10.000	6.000	5.000	3.120	2.496	2.321	3.045
31 X1794	4.500	0.250	0.600	4.250	2.540	2.677	2.481	3.061
32 X1739	17.000	7.500	2.250	2.250	2.859	3.483	2.979	3.206
33 X1799	8.750	6.750	4.000	2.000	2.767	4.887	2.280	4.075

APPENDIX #A COMPOSITED RAW DATA PRINTOUT THYMIDINE PAGE #3

	ANIMAL NAME	RCAM	RCAD	LCAM	LCAD	RAPEX	LAPEX	TIMSAC
1	B1294	0.000	0.000	0.000	0.000			14.50
2	B1107	0.000	0.127	0.000	0.000			11.00
3	B1211	0.728	0.413	0.595	0.406			11.75
4	C1302	2.918	1.057	2.385	1.661	82	85	11.50
5	C1819	0.405	0.177	0.000	0.103	82	85	13.75
6	C1462	0.000	0.290	0.616	0.000			14.00
7	C1835	0.773	1.270	3.987	0.642		80	23.25
8	C1367	1.025	1.090	2.092	0.785		99	13.50
9	C1423	2.930	0.671	0.551	0.669	96	104	00.50
10	X1520	0.398	0.207	1.173	1.181			19.50
11	X1757	0.087	0.922	0.348	0.160	80	82	19.75
12	X1068	1.219	1.140	2.150	1.564			11.75
13	X1985	3.928	2.410	3.043	1.005			11.29
14	X1466	0.761	0.116	0.380	1.830			15.75
15	X1625	0.382	1.490	2.082	3.623	89		00.25
16	X1040	2.849	7.796	1.869	6.635		92	13.00
17	X1958	0.881	3.155	1.754	2.240		75	16.75
18	X1160	3.188	2.751	0.880	2.623			09.50
19	X1343	2.268	1.489	0.949	3.514			16.50
20	X1083	1.943	2.438	2.762	4.347			18.00
21	X1424	9.153	0.447	2.213	0.333			06.92
22	X1978	1.542	4.546	0.804	1.633			11.50
23	X1095	4.419	2.436	3.347	1.976			11.75
24	X1659	8.171	5.885	3.125	1.047	70	80	00.50
25	X1679	1.180	1.542	0.865	1.980		94	00.75
26	X1078	1.599	5.811	0.469	2.694			09.00
27	X1773	3.603	6.297	1.822	0.818			20.25
28	X1290	0.090	0.000	0.000	0.000			10.08
29	X1065	1.671	0.415	1.558	0.484			10.33
30	X1956	3.606	4.007	2.586	1.642		80	16.75
31	X1794	1.772	0.093	0.242	1.389			11.83
32	X1739	5.946	2.154	0.755	0.702			24.00
33	X1799	3.163	1.381	1.754	0.491			14.50

APPENDIX #A COMPOSITED RAW DATA PRINTOUT URIDINE PAGE #1

ANIMAL NAME	TIME	WTINJ	WTSAC	WTANA	GROWTH	WTLOSS	RLIGW
67 B3715	24.	75.7	75.7	74.5	1.2	0.0	555.333
68 B3108	48.	79.2	77.7	77.7	1.5	-1.5	000.000
69 B3041	72.	97.5	96.6	78.9	18.6	-0.9	527.333
70 C3957	12.	80.8	81.1	86.4	-5.6	0.3	000.000
71 C3066	24.	73.2	72.4	66.8	6.4	-0.8	000.000
72 C3859	36.	90.2	87.8	82.5	7.7	-2.4	000.000
73 C3075	48.	80.9	80.6	75.1	5.8	-0.3	000.000
74 C3540	60.	91.4	89.0	79.3	12.1	-2.4	000.000
75 C3924	72.	81.6	79.9	65.2	16.4	-1.7	000.000
76 X3974	6.	72.7	71.3	72.4	0.3	-1.4	443.333
77 X3881	6.	66.8	66.0	65.5	1.3	-0.8	564.667
78 X3140	12.	70.3	69.1	72.8	-2.5	-1.2	443.333
79 X3529	12.	64.1	63.3	64.1	0.0	-0.8	368.667
80 X3171	18.	77.6	77.7	72.3	5.3	0.1	536.667
81 X3652	18.	79.0	77.7	76.5	2.5	-1.3	448.000
82 X3374	24.	77.0	76.2	76.0	1.0	-0.8	513.333
83 X3508	24.	73.6	73.2	70.5	3.1	-0.4	602.000
84 X3240	30.	67.7	66.3	66.3	1.4	-1.4	490.000
85 X3161	30.	84.9	83.3	32.0	2.9	-1.6	518.000
86 X3365	36.	70.2	69.0	69.4	0.8	-1.2	536.667
87 X3913	36.	84.3	81.8	76.7	7.6	-2.5	555.333
88 X3471	42.	85.5	84.1	73.6	11.9	-1.4	527.333
89 X3724	42.	83.0	81.8	71.5	11.5	-1.2	606.667
90 X3728	48.	88.5	87.0	78.1	10.4	-1.5	513.333
91 X3387	48.	88.0	87.2	76.0	12.0	-0.8	490.000
92 X3196	54.	86.4	83.6	74.5	11.9	-2.8	448.000
93 X3472	54.	95.4	93.2	81.9	13.5	-2.2	494.667
94 X3590	60.	93.1	90.1	73.1	20.0	-3.0	518.000
95 X3992	60.	86.5	83.8	76.5	10.0	-2.7	560.000
96 X3248	66.	94.3	94.3	78.4	15.9	0.0	452.667
97 X3994	66.	92.2	91.5	75.5	16.7	-0.7	504.000
98 X3099	72.	86.3	85.2	72.6	13.7	-1.1	508.667
99 X3843	72.	79.4	78.7	64.8	14.6	-0.7	574.000

APPENDIX #A COMOSITED RAW DATA PRINTOUT URIDINE PAGE #2

ANIMAL NAME	RDRM	RDRD	LDRM	LDRD	RCWM	RCWD	LCWM	LCWD
67 B3715	0.888	0.838	1.205	1.436	1.422	3.314	2.351	3.113
68 B3108	1.158	0.288	0.588	0.360	2.709	3.405	3.073	3.862
69 B3041	0.206	0.242	0.569	0.649	3.501	3.247	2.806	3.372
70 C3957	0.827	0.655	0.787	0.812	2.099	3.644	2.470	4.261
71 C3066	0.695	0.675	0.863	0.786	2.662	4.212	2.250	3.366
72 C3859	0.604	0.604	0.650	0.787	2.617	2.998	2.818	2.745
73 C3075	0.584	0.584	1.038	0.801	2.426	3.078	2.568	3.345
74 C3540	0.650	0.559	2.084	1.883	2.080	3.248	2.546	3.196
75 C3924	0.888	0.523	1.064	0.534	2.890	3.157	2.285	3.775
76 X3974	1.038	0.936	1.086	1.160	1.833	4.012	2.247	2.971
77 X3881	0.518	0.939	0.681	0.579	1.257	3.834	2.310	3.515
78 X3140	0.838	0.508	1.126	0.777	3.429	3.317	2.696	3.060
79 X3529	1.136	1.236	1.610	1.834	2.644	3.802	1.995	2.867
80 X3171	1.782	0.700	0.513	0.544	2.972	4.113	2.767	3.453
81 X3652	0.937	0.762	1.015	1.113	1.921	2.814	2.034	2.916
82 X3374	1.236	1.162	1.584	1.559	2.352	3.088	2.599	3.929
83 X3508	0.775	0.888	0.818	1.211	1.572	3,942	2.365	2.713
84 X3240	0.849	0.842	1.013	1.010	1.896	3,039	2.363	3.236
85 X3161	0.862	1.011	1.109	0.650	1.653	3,643	3.040	3.140
86 X3365	0.800	1.136	1.015	1.110	2.313	3.063	1.583	3.523
87 X3913	0.680	0.960	0.554	0.554	1.684	3.500	2.522	3.134
88 X3471	1.685	0.827	1.236	1.050	2.381	2.131	2.328	3.397
89 X3724	0.913	0.987	1.837	1.013	1.926	2.476	3.458	3.825
90 X3728	1.585	0.719	0.862	0.987	2.357	2.886	2.527	3.627
91 X3387	1.187	0.890	2.185	1.883	2.184	2.762	2.581	3.452
92 X3196	1.134	0.630	0.888	0.887	2.161	4.125	2.885	3.148
93 X3472	1.510	1.036	1.236	1.062	2.274	3.089	2.052	4.059
94 X3590	1.074	1.135	1.183	1.883	2.278	2.790	2.501	4.604
95 X3992	0.987	0.812	0.685	0.544	3.068	3.867	3.097	3.754
96 X3248	0.886	0.729	0.984	1.086	1.695	4.152	2.976	2.717
97 X3994	1.062	0.990	0.736	0.665	2.977	4.202	2.654	2.867
98 X3099	1.032	0.579	1.038	0.573	3.297	2.922	3.457	3.230
99 X3843	1.089	1.187	0.935	0.660	2.957	2.664	2.940	3.541

APPENDIX #A COMPOSITED RAW DATA PRINTOUT URIDINE PAGE #3

ANIMAL NAME	RDOTM	RDOTD	LDOTM	LDOTD	RAPEX	LAPEX	TIMSAC
67 B3715	1.262	2.776	2.832	4.472			18.25
68 B3108	3.138	0.982	1.808	1.390			16.25
69 B3041	0.723	0.787	1.597	2.187			16.00
70 C3957	1.736	2.386	1.943	3.459	86	88	17.50
71 C3066	1.851	2.844	1.941	2.645	78	75	17.75
72 C3859	1.580	1.812	1.832	2.160	78		10.00
73 C3075	1.417	1.796	2.667	2.678		61	15.75
74 C3540	1.352	1.815	5.306	6.019	90		10.25
75 C3924	2.567	1.653	2.431	2.015	81	88	15.50
76 X3974	1.904	3.757	2.440	3.445	85	82	11.75
77 X3881	0.652	3.599	1.572	2.037			00.25
78 X3140	2.872	1.684	3.035	2.377			18.50
79 X3529	3.005	4.700	3.212	5.258	92	100	20.00
80 X3171	5.297	2.880	1.420	1.879		83	11.00
81 X3652	1.799	2.144	2.064	3.245			11.50
82 X3374	2.908	3.588	4.117	6.123		94	16.75
83 X3508	1.219	3,501	1.935	3.286		98	17.25
84 X3240	1.611	2.560	2.394	3.266	75		13.00
85 X3161	1.425	3.682	3.373	2.041	80	61	13.50
86 X3365	1.851	3.480	1.607	3.912		83	09.00
87 X3913	1.145	3.361	1.397	1.737	88		09.50
88 X3471	4.011	1.763	2.877	3.566	77	81	14.00
89 X3724	1.759	2.443	6.351	3.875			14.50
90 X3728	3.735	2.075	2.179	3.582		102	14.75
91 X3387	2.593	2.457	5.640	6.501		100	19.00
92 X3196	2.452	2.597	2.561	2.791	93		11.50
93 X3472	3.434	3.201	2.537	4.310			24.00
94 X3590	2.446	3.165	2.960	8.669	99	103	09.25
95 X3992	3.030	3.140	2.122	2.040	100	90	09.75
96 X3248	1.502	3.029	2.929	2.950		87	14.25
97 X3994	3.163	4.158	1.955	1.907	95	94	14.75
98 X3099	3.402	1.693	3.590	1.852		92	14.50
99 X3843	3,221	3.162	2.749	2.338	95		15.00

APPENDIX #A COMPOSITED RAW DATA PROLINE PAGE #1

ANIMAL NAME	TIME	WTINJ	WTSAC	WTANA	GROWTH	WTLOSS	RLIGW
34 B2818	24.	65.5	66.3	65.0	0.5	0.8	532.000
35 B2257	48.	-0.0	85.5	72.0	-0.0	-0.0	564.667
36 B2735	72.	-0.0	100.7	86.1	-0.0	-0.0	000.000
37 C2307	12.	64.9	64.6	63.6	1.4	-0.3	000.000
38 C2688	24.	89.5	88.4	86.6	2.9	-1.1	000.000
39 C2281	36.	92.3	90.0	83.3	9.0	-2.3	000.000
40 C2562	48.	81.7	81.0	67.7	14.0	-0.7	000.000
41 C2765	60.	96.1	94.0	80.8	15.3	-2.1	000.000
42 C2522	72.	75.7	75.3	64.6	11.1	-0.4	000.000
43 X2252	6.	86.8	84.5	86.4	0.4	-2.3	499.333
44 X2928	6.	75.5	76.4	80.0	-4.5	0.9	508.667
45 X2187	12.	78.3	76.9	81.1	-2.8	-1.4	504.000
46 X2761	12.	67.2	68.3	72.5	-5.3	1.1	574.000
47 X2004	18.	69.2	66.3	69.2	0.0	-2.9	555.333
48 X2588	18.	65.7	65.0	64.2	1.5	-0.7	555.333
49 X2878	24.	68.0	67.8	70.5	-2.5	-0.2	574.000
50 X2328	24.	82.2	82.0	74.0	8.2	-0.2	553.000
51 X2158	30.	87.3	86.2	81.6	5.7	-1.1	639.333
52 X2038	30.	74.5	73.6	65.3	9.2	-0.9	602.000
53 X2551	36.	71.6	70.5	55.1	16.5	-1.1	653.333
54 X2854	36.	69.5	68.8	64.6	4.9	-0.7	490.000
55 X2024	42.	91.6	90.1	76.4	15.2	-1.5	480.667
56 X2486	42.	98.5	98.6	93.0	5.5	0.1	518.000
57 X2967	48.	87.3	86.4	80.4	6.9	-0.9	527.333
58 X2946	48.	92.1	89.3	77.5	14.6	-2.8	462.000
59 X2569	54.	83.1	83.0	78.3	4.8	-0.1	527.333
60 X2117	54.	101.9	100.9	88.8	13.1	-1.0	588.000
61 X2360	60.	93.2	90.0	84.5	8.7	-3.2	476.000
62 X2168	60.	91.2	90.0	75.7	15.5	-1.2	592.667
63 X2165	66.	75.8	74.9	64.9	10.9	-0.9	532.000
64 X2856	66.	71.4	71.0	64.0	7.4	-0.4	494.667
65 X2310	72.	72.2	70.6	64.0	8.2	-1.6	639.333
66 X2653	72.	101.3	100.3	85.5	15.8	-1.0	499.333

APPENDIX #A COMPOSITED RAW DATA PROLINE PAGE #2

	ANIMAL NAME	RDRM	RDRD	LDRM	LDRD	RCWM	XRCWD	XLCWM	XLCWD
34	B2818	8.396	6.846	4.614	6.644	2.141	2.987	3.423	3.818
35	B2257	8.423	6.921	7.864	7.359	2.035	4.157	3.630	2.981
36	B2735	6.605	1.317	2.467	3.041	2.881	4.654	3.006	2.928
37	C2307	22.288	23.193	23.478	24.098	3.295	3.382	3.890	2.986
38	C2688	16.473	22.288	20.143	24.098	2.515	3.455	2.774	5.095
39	C2281	20.143	22.288	21.383	21.383	2.714	4.492	2.312	3.610
40	C2562	19.523	19.389	16.188	21.048	2.969	4.133	3.111	3.514
41	C2765	18.668	25.003	21.333	18.048	3.414	2.905	3.282	3.013
42	C2522	20.755	18.048	16.071	15.903	3.392	3.878	3.096	3.630
43	X2252	14.395	23.193	17.294	16.808	3.128	3.551	3.447	4.427
44	X2928	13.657	22.288	12.711	13.196	2.021	4.140	3.096	3.869
45	X2187	6.417	5.656	5.583	6.599	2.020	3.104	3.779	3.043
46	X2761	9.062	18.048	15.568	15.149	1.683	2.789	3.330	3.550
47	X2004	8.794	15.006	8.664	16.058	2.897	3.925	3.225	4.726
48	X2588	12.752	16.594	18.953	22.288	1.521	3.075	3.305	3.080
49	X2878	8.800	13.909	17.093	21.383	2.042	3.486	2.790	3.440
50	X2328	10.442	12.576	12.401	12.886	2.538	3.396	3.310	3.544
51	X2158	22.288	23.193	18.484	16.523	2.614	4.903	2.837	5.007
52	X2038	10.522	19.104	13.331	15.283	1.745	3.316	2.571	3.304
53	X2551	12.711	18.199	16.808	21.668	1.952	3.627	2.644	3.510
54	X2854	20.478	15.785	13.155	22.288	2.819	2.831	2.389	3.041
55	X2024	9.056	15.166	0.168	0.168	3.137	3.955	2.674	4.695
56	X2486	16.808	17.579	7.861	21.668	3.551	3.133	1.261	3.996
57	X2967	6.599	3.041	10.448	9.104	1.678	3.219	3.086	3.545
58	X2946	0.168	0.168	2.783	3.041	2.471	4.374	2.200	3.752
59	X2569	14.663	16.523	18.048	15.268	2.746	4.451	3.067	4.738
60	X2117	11.337	22.288	11.647	16.959	3.035	4.579	2.591	4.602
61	X2360	17.000	24.098	24.098	23.193	2.939	4.004	2.911	3.439
62	X2168	13.599	19.858	13.775	15.434	3.255	4.426	3.127	3.980
63	X2165	18.048	15.903	12.711	12.711	2.919	3.119	2.720	2.899
64	X2856	18.618	15.719	14.529	19.858	3.030	3.387	2.818	3,608
65	X2310	19.238	13.197	23.193	24.098	3.020	3.065	3.243	3.412
66	X2653	20.470	20.889	12.581	13.919	3.207	2.604	3.305	3.317

APPENDIX #A COMPOSITED RAW DATA PROLINE PAGE #3

	ANIMAL NAME	RDOTM	RDOTD	LDOTM	LDOTD	RAPEX	LAPEX	TIMSAC
34	B2818	17.971	20.445	15.790	25.365			18.25
35	B2257	17.142	28.770	28.542	21.934			16.25
36	B2735	19.029	6.131	7.415	8.906			16.00
37	C2307	73.430	78.436	91.320	71.954			17.50
38	C2688	41.427	76.998	55.868	122.787		75	15.75
39	C2281	54.664	100.108	49.437	77.199	125		10.00
40	C2562	57.972	80.132	50.360	73.966	87	93	15.75
41	C2765	63.735	72.639	70.015	54.384		103	10.25
42	C2522	70.390	69.994	49.749	57.723		91	15.50
43	X2252	45.022	82.352	59.620	74.416	102		11.75
44	X2928	27.604	92.272	39.350	51.062			00.25
45	X2187	12.963	17.555	21.099	20.078	100	103	18.50
46	X2761	15.252	50.343	51.839	53.779			20.00
47	X2004	25.473	58.901	27.942	75.897		75	11.00
48	X2588	19.394	51.027	62.642	68.636			11.50
49	X2878	17.970	48.492	47.697	73.565	80		16.25
50	X2328	26.501	42.713	41.047	45.663		81	17.25
51	X2158	58.261	113.713	52.436	82.725	90		13.00
52	X2038	18.357	63.352	34.277	50.493		73	13.50
53	X2551	24.813	66.001	44.437	76.043			09.00
54	X2854	57.724	44.687	31.425	67.782			09.58
55	X2024	28.410	59.979	0.449	0.789	105		14.00
56	X2486	59.693	55.077	9,916	86.583		72	14.25
57	X2967	11.074	9.788	32.239	32.272	79	85	14.75
58	X2946	0.415	0.735	6.122	11.411		104	15.25
59	X2569	40.265	73.546	55.357	72.345		70	19.75
60	X2117	34.405	102.048	30.173	78.052			24.00
61	X2360	49.969	96.480	70.154	79.754			20.00
62	X2168	44.258	87.899	43.075	61.428		102	09.75
63	X2165	52.685	49.607	34.577	36.847			14.25
64	X2856	56.407	53.241	40.938	71.646			14.75
65	X2310	58.104	40.450	75.218	82.224			14.50
66	X2653	65.654	54.390	41.582	46.168			15.00

APPENDIX B

TABLES

Key to Tables

The right side of the experimental animals is the treated side (where elastic has been placed). The left side of the experimental animals is termed the untreated side--an in-animal control. Therefore in experimental animals,

"Pressure" side = Upper RIGHT first molar, MESIAL surface of root;

"Tension" side = Upper RIGHT first molar, DISTAL surface of root;

"Pressure control" side = Upper LEFT first molar, MESIAL surface of root;

"Tension control" side = Upper LEFT first molar DISTAL surface of root.

The control animals are completely untreated (free of elastics). In designating control animal areas, the word "analog" has been substituted for "side."

Thus, in control animals

"Pressure" analog = Upper RIGHT first molar, MESIAL surface of root;

"Tension" analog = Upper RIGHT first molar DISTAL surface of root;

"Pressure control" analog = Upper LEFT first molar MESIAL surface of root;

"Tension control" analog = Upper LEFT first molar DISTAL surface of root.

Table 8-1. Replicating Cells per Unit of PDL Area
(In Cells/100^2 sq. u. - "CA" Variables)

		Group "A"	vs	Group "B"	Probability	T-RATIO	d.f.	Group "A" Statistics			Group "B" Statistics		
								mean	sigma	n	mean	sigma	n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	not signf.	1.3008	28	2.66	2.38	24	1.34	1.27	6
		"Tension" Side		"Tension" Analog	A>B P <.10	1.8495	28	2.46	2.21	24	0.76	0.45	6
		"Pressure Control" Side		"Pressure Control" Analog	not signf.	-0.1324	28	1.54	0.99	24	1.61	1.50	6
		"Tension Control" Side		"Tension Control" Analog	A>B P <.10	1.8519	28	1.83	1.52	24	0.64	0.59	6
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	A>B P <.05	2.6742	23	1.1204	2.0525	0.4190			
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	1.5167	23	0.6257	2.0210	0.4125			
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	not signf.	0.3613	23	0.2038	2.7631	0.5640			
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	not signf.	-0.8500	23	-0.2909	1.6764	0.3422			
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-0.3444	5	-0.2633	1.8729	0.7646			
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	0.6836	5	0.1158	0.4150	0.1694			
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	not signf.	1.2135	5	0.5827	1.1762	0.4802			
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	not signf.	1.8314	5	0.9618	1.2864	0.5252			

Table 8-2. Total Replicating Cells per PDL Field
(in Cells per Photograph - "CC" Variables)

		Group "A"	vs	Group "B"	Probability	T-RATIO	d.f.	Group "A" Statistics mean	Group "A" Statistics sigma	Group "A" Statistics n	Group "B" Statistics mean	Group "B" Statistics sigma	Group "B" Statistics n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	not signf.	1.3465	28	6.78	5.96	24	3.36	3.16	6
		"Tension" Side		"Tension" Analog	not signf.	1.6084	28	9.33	10.00	24	2.65	1.70	6
		"Pressure Control" Side		"Pressure Control" Analog	not signf.	-0.0267	28	4.25	3.11	24	4.30	3.80	6
		"Tension Control" Side		"Tension Control" Analog	A>B P < .10	1.8122	28	6.98	6.36	24	2.15	2.16	6
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	A>B P < .10	2.0343	23	2.5313	6.0961	1.2444			
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	1.4645	23	2.3548	7.8770	1.6079			
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	not signf.	-1.1928	23	-2.5465	10.4591	2.1350			
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	B>A P <.05	-2.1300	23	-2.7230	6.2630	1.2784			
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-0.5283	5	-0.9305	4.3142	1.7613			
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	0.6731	5	0.5028	1.8298	0.7470			
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	not signf.	0.6259	5	0.7083	2.7723	1.1318			
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	not signf.	1.6178	5	2.1417	3.2427	1.3238			

Table 8-3. Synthetic Activity per Unit Area - Proline
(In Hundreds of Counts/100^2 sq. u. - "DR" Variables)

		Group "A"	vs	Group "B"	Probability	T-RATIO	d.f.	Group "A" Statistics			Group "B" Statistics		
								mean	sigma	n	mean	sigma	n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	B>A P < .01	-2.8805	28	13.16	5.36	24	19.64	1.97	6
		"Tension" Side		"Tension" Analog	B>A P < .05	-2.1489	28	16.17	6.11	24	21.70	2.55	6
		"Pressure Control" Side		"Pressure Control" Analog	B>A P < .05	-2.6091	28	13.41	5.72	24	19.77	3.01	6
		"Tension Control" Side		"Tension Control" Analog	B>A P < .10	-1.9276	28	15.65	6.23	24	20.76	3.28	6
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	not signf.	-0.2344	23	mean	sigma	sigma M.D.			
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	0.4147	23	-0.2485	5.1938	1.0602			
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	B>A P < .01	-3.0336	23	0.5179	6.1182	1.2489			
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	B>A P < .01	-2.8330	23	-3.0024	4.8486	0.9897			
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-0.0906	5	-2.2360	3.8665	0.7892			
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	0.6909	5	-0.1243	3.3614	1.3723			
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	not signf.	-1.4415	5	0.9385	3.3273	1.3584			
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	not signf.	-0.8186	5	-2.0598	3.5001	1.4290			
								-0.9970	2.9833	1.2179			

Table 8-4. Total Synthetic Activity per PDL Field - Proline
(In Hundreds of Counts/Photograph - "DOT" Variables)

		Group "A"	vs	Group "B"	Probability	T-RATIO	d.f.	Group "A" Statistics			Group "B" Statistics		
								mean	sigma	n	mean	sigma	n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	B>A P < .01	-3.0603	28	35.44	18.84	24	60.27	11.66	6
		"Tension" Side		"Tension" Analog	B>A P < .10	-1.7782	28	58.94	27.80	24	79.72	10.67	6
		"Pressure Control" Side		"Pressure Control" Analog	B>A P < .05	-2.5468	28	39.73	18.74	24	61.12	16.74	6
		"Tension Control" Side		"Tension Control" Analog	not signf.	-1.6474	28	58.32	23.83	24	76.34	24.54	6
								mean		sigma		sigma M.D.	
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	not signf.	-0.9403	23	-4.2891		22.3454		4.5612	
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	0.1251	23	0.6246		24.4538		4.9916	
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	B>A P < .01	-4.4956	23	-23.4990		25.6075		5.2271	
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	B>A P < .01	4.7272	23	-18.5852		19.2606		3.9316	
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-0.1429	5	-0.8552		14.6626		5.9860	
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	0.3318	5	3.3823		24.9667		10.1926	
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	B>A P < .05	-2.6171	5	-19.4482		18.2024		7.4311	
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	not signf.	-1.1665	5	-15.2107		31.9402		13.0395	

Table 8-5. Synthetic Activity per Unit Area - Uridine
(In Hundreds of Counts/100^2 sq. u. - "DR" Variables)

		Group "A"	vs	Group "B"	Prob-ability	T-RATIO	d.f.	Group "A" Statistics			Group "B" Statistics		
								mean	sigma	n	mean	sigma	n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	A>B P < .05	2.7313	28	10.7	3.1	24	7.1	1.2	6
		"Tension" Side		"Tension" Analog	A>B P < .01	3.6434	28	9.0	2.0	24	6.0	0.6	6
		"Pressure Control" Side		"Pressure Control" Analog	not signf.	-0.0028	28	10.8	4.0	24	0.8	15.2	6
		"Tension Control" Side		"Tension Control" Analog	not signf.	0.4211	28	10.2	4.2	24	9.3	4.8	6
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	not signf.	-0.1437	23	mean	sigma	sigma M.D.			
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	-1.4971	23	-0.139	4.743	0.968			
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	A>B P < .05	2.1166	23	-1.164	3.808	0.777			
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	not signf.	1.0265	23	1.664	3.852	0.786			
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-1.6734	5	0.640	3.052	0.623			
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	-1.6678	5	-3.730	5.460	2.229			
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	not signf.	1.8567	5	-3.338	4.903	2.002			
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	not signf.	1.5432	5	1.080	1.425	0.582			
								1.472	2.336	0.954			

Table 8-6. Total Synthetic Activity per PDL Field - Uridine
(In Hundreds of Counts/Photograph - "DOT" Variables)

		Group "A"	vs	Group "B"	Probability	T-RATIO	d.f.	Group "A" Statistics mean	sigma	n	Group "B" Statistics mean	sigma	n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	not signf.	1.6897	28	25.2	10.8	24	17.5	4.4	6
		"Tension" Side		"Tension" Analog	A>B P < .01	2.7894	28	29.9	07.9	24	20.5	4.6	6
		"Pressure Control" Side		"Pressure Control" Analog	not signf.	0.1883	28	29.0	12.1	24	26.9	13.2	6
		"Tension Control" Side		"Tension Control" Analog	not signf.	0.3857	28	34.6	17.1	24	31.6	14.9	6
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	not signf.	-0.8265	23	mean	sigma	sigma M.D.			
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	-1.2598	23	-2.742	16.251	3.317			
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	not signf.	1.5552	23	-4.653	18.095	3.694			
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	B>A P < .10	-2.0365	23	-4.743	14.940	3.050			
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-1.4758	5	-6.655	16.008	3.268			
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	-1.7236	5	-9.362	15.538	6.343			
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	not signf.	-1.1320	5	-11.117	15.799	6.450			
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	not signf.	-1.7484	5	3.005	6.502	2.655			
								-4.760	6.669	2.722			

Table 8-7. PDL Area
(In 100^2 sq. u. - "CW" Variables - Opposite Surfaces of Root Considered Separately

		Group "A"	vs	Group "B"	Probability	T-RATIO	d.f.	Group "A" Statistics			Group "B" Statistics		
								mean	sigma	n	mean	sigma	n
Between Animal Differences Experimental Animals vs. Control Animals		"Pressure" Side		"Pressure" Analog	not signf.	-0.9326	88	2.48	0.56	72	2.62	0.53	18
		"Tension" Side		"Tension" Analog	not signf.	0.0701	88	3.62	0.64	72	3.49	0.59	18
		"Pressure Control" Side		"Pressure Control" Analog	not signf.	0.1846	88	2.75	0.59	72	2.72	0.52	18
		"Tension Control" Side		"Tension Control" Analog	not signf.	0.7944	88	3.62	0.64	72	3.49	0.59	18
Within Animal Differences Experimental Animals Only	Contra-Lateral	"Pressure" Side		"Pressure Control" Side	B>A P .05	-2.6202	71	mean	sigma	sigma M.D.			
	Contra-Lateral	"Tension" Side		"Tension Control" Side	not signf.	-0.8059	71	-0.2609	0.8448	0.0996			
	Ipsi-Lateral	"Pressure" Side		"Tension" Side	B>A P < .01	-10.0431	71	-0.0899	0.9467	0.1116			
	Ipsi-Lateral	"Pressure Control" Side		"Tension Control" Side	B>A P < .01	-9.3091	71	-1.0444	0.8824	0.1040			
Within Animal Differences Control Animals Only	Contra-Lateral	"Pressure" Analog		"Pressure Control" Analog	not signf.	-0.7571	17	-0.8734	0.7961	0.0938			
	Contra-Lateral	"Tension" Analog		"Tension Control" Analog	not signf.	0.1542	17	-0.0956	0.5355	0.1262			
	Ipsi-Lateral	"Pressure" Analog		"Tension" Analog	B>A P < .01	-4.7796	17	0.0282	0.7764	0.1830			
	Ipsi-Lateral	"Pressure Control" Analog		"Tension Control" Analog	B>A P < .01	-3.7581	17	-0.8940	0.7936	0.1870			
								-0.7702	0.8695	0.2050			

TABLE 8-8 TOTAL PDL AREA*

Group A	vs.	Group B	Sig. Level	T-ratio	df	....Group A....			....Group B....		
						$\bar{x}$	s	n	$\bar{x}$	s	n
Experimental Animals Rt. side (treated		Control Animals Rt. side	not signf.	-0.4907	88	6.01	1.00	72	6.14	0.73	18
Experimental Animals Lt. side (untreated)		Control Animals Lt. side	not signf.	-0.6744	88	6.36	0.94	72	6.20	0.70	18
Experimental Animals Rt. side (treated)		Experimental Animals Lt. side (untreated)	B>A $\alpha = .05$	-2.1857	71	6.01	1.00	72	6.36	0.94	72
Control Animals Rt. side		Control Animals Lt. side	not signf.	-0.2909	17	6.14	0.73	18	6.20	0.70	18

*Total PDL area is considered to be the sum of the areas of the PDL in the pair of photographs taken of each histologic slide. Since one of these photographs represents the mesial surface of the first molar root, and the other photograph represents its distal surface, the summed dimensions give a measure of the width of the periodontal space in which the root lies.

TABLE A

CORRELATION MATRIX--EXPERIMENTAL ANIMALS (N = 24)

TOTAL CELLS PER PDL FIELD

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.220	.218	-.208
R. Distal	.220		.085	.617 (.01)
L. Mesial	.218	.085		.275
L. Distal	-.208	.617 (.01)	.275	.275

TABLE A'

CORRELATION MATRIX--CONTROL ANIMALS (N = 6)

TOTAL CELLS PER PDL FIELD

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.481	.240	.862 (.05)
R. Distal	.481		.906 (.05)	.572
L. Mesial	.240	.906 (.05)		.522
L. Distal	.862 (.05)	.572	.522	

TABLE B

CORRELATION MATRIX--EXPERIMENTAL ANIMALS (N = 24)

REPLICATING CELLS PER UNIT OF PDL AREA

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.274	.512 (.05)	-.176
R. Distal	.274		.304	.462 (.05)
L. Mesial	.512 (.05)	.304		.163
L. Distal	-.176	.462 (.05)	.163	

TABLE B'

CORRELATION MATRIX--CONTROL ANIMALS (N = 6)

REPLICATING CELLS PER UNIT OF PDL AREA

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.384	.092	.786 (.10)
R. Distal	.384		.916 (.05)	.717
L. Mesial	.092	.916 (.05)		.526
L. Distal	.786 (.10)	.717	.526	

TABLE C

CORRELATION MATRIX--EXPERIMENTAL ANIMALS (N = 24)

COMPARATIVE FIELD DENSITY--PROLINE GRAINS

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.650 (.01)	.562 (.01)	.601 (.01)
R. Distal	.650 (.01)		.514 (.05)	.509 (.05)
L. Mesial	.562 (.01)	.514 (.05)		.794 (.01)
L. Distal	.601 (.01)	.509 (.05)	.794 (.01)	

TABLE C'

CORRELATION MATRIX--CONTROL ANIMALS (N = 6)

COMPARATIVE FIELD DENSITY--PROLINE GRAINS

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.122	-.136	-.366
R. Distal	-.122		-.495	-.178
L. Mesial	-.136	-.495		.892 (.05)
L. Distal	-.336	-.178	.892 (.05)	

TABLE D

CORRELATION MATRIX--EXPERIMENTAL ANIMALS (N = 24)

TOTAL GRAIN COUNTS PER PDL FIELD--PROLINE

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.451 (.05)	.293	.505 (.05)
R. Distal	.451 (.05)		.375 (.10)	.561 (.01)
L. Mesial	.293	.375 (.10)		.614 (.01)
L. Distal	.505 (.05)	.561 (.01)	.614 (.01)	

TABLE D'

CORRELATION MATRIX--CONTROL ANIMALS (N = 6)

TOTAL GRAIN COUNTS PER PDL FIELD--PROLINE

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.030	-.398	-.547
R. Distal	-.030		-.373	-.048
L. Mesial	-.398	-.373		.894 (.05)
L. Distal	-.547	-.048	.894 (.05)	

TABLE E

CORRELATION MATRIX--EXPERIMENTAL ANIMALS

COMPARATIVE FIELD DENSITY--URIDINE GRAINS

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.097	.121	.141
R. Distal	-.097		.297	.424 (.05)
L. Mesial	.121	.297		.722 (.01)
L. Distal	.141	.424 (.05)	.722 (.01)	

TABLE E'

CORRELATION MATRIX--CONTROL ANIMALS

COMPARATIVE FIELD DENSITY--URIDINE GRAINS

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.184	.142	-.131
R. Distal	-.184		.876 (.05)	.369
L. Mesial	.142	.876 (.05)		.553
L. Distal	-.131	.369	.553	

TABLE F

CORRELATION MATRIX--EXPERIMENTAL ANIMALS

TOTAL GRAIN COUNTS PER PDL FIELD--URIDINE

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.265	-.004	.029
R. Distal	-.265		-.232	.105
L. Mesial	-.004	-.232		.444 (.05)
L. Distal	.029	.105	.444 (.05)	

TABLE F'

CORRELATION MATRIX--CONTROL ANIMALS

TOTAL GRAIN COUNTS PER PDL FIELD--URIDINE

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.328	.515	-.837 (.05)
R. Distal	-.328		-.246	.177
L. Mesial	.515	-.246		-.168
L. Distal	-.837 (.05)	.177	-.168	

TABLE G

CORRELATION MATRIX--EXPERIMENTAL ANIMALS (N = 72)

AREA OF PDL PER PHOTOGRAPHIC FIELD

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		.133	-.063	-.055
R. Distal	.133		-.050	-.085
L. Mesial	-.063	-.050		.163
L. Distal	-.055	-.085	.163	

TABLE G'

CORRELATION MATRIX--CONTROL ANIMALS (N = 18)

AREA OF PDL PER PHOTOGRAPHIC FIELD

	R. Mesial	R. Distal	L. Mesial	L. Distal
R. Mesial		-.085	.487 (.05)	-.160
R. Distal	-.086		-.268	.070
L. Mesial	.487 (.05)	-.268		-.209
L. Distal	-.160	.070	-.209	

TABLE H

MEASURES OF THE DEGREE OF BILATERAL SYMMETRY
IN THE DISTANCE BETWEEN THE APICES OF THE
APPROXIMATING ROOTS OF THE FIRST
AND SECOND MOLARS

A. Experimental Animals (Dimensions are in 10's of u)

Right Side (treated)	Left Side (untreated)
$\bar{x}$ = 88.7	$\bar{x}$ = 87.3
s = 10.0	s = 11.5
n = 20	n = 30

B. Control Animals

Right Side	Left Side
$\bar{x}$ = 88.5	$\bar{x}$ = 89.2
x = 14.0	s = 9.4
n = 10	n = 13

TABLE H'

MEASURES OF ASSOCIATION OF INTER APICAL DISTANCE
(RIGHT SIDE VS. LEFT SIDE)

Experimental animals	r = plus .743	Significant at .01 level
Control animals	r = plus .949	Significant at .01 level

APPENDIX C

THE NATURE OF THE PERIODONTAL LIGAMENT

The periodontal ligament intervenes between the tooth root and the alveolar bone. It is the mechanism by which the tooth is attached to the bone and also subserves the function of damping mechanical forces transmitted through the tooth to the bone.

The mechanically functional portions of the PDL are elaborated and organized by its cellular elements. In the body of the ligament, the most prevalent cell types are fibroblasts or fibrocytes, which are morphologically indistinguishable from each other by conventional histologic procedures. In apposition to the alveolar wall, osteoblasts and occasional osteoclasts are found. This portion of the PDL is osteogenic and, indeed, some authors feel that the entire PDL is best considered a specialized periosteum. (9) Adjacent to the tooth surface, cementoblasts are found.

The structural elements of the PDL include fibers and ground substance. In addition, certain specialized arteriovenous shunts, the glomeruli of Ishikawa, are found. These are believed to play an auxiliary shock absorbing role.

The Fibers of the PDL

The fibers of the PDL are its most striking histologic feature. They were first described by Tomes and early were reputed to have the role of shock absorbers. Since the fibers of the PDL are composed almost exclusively of collagen, a consideration of the chemistry of that material is in order.

Collagen is a protein composed largely of glycine, proline, and hydroxyproline with important though small amounts of lycine, and hydroxylycine. Its molecular configuration is helical, consisting of three strands, each with a left rotation. In turn, the three strands are coiled about each other with a right rotation. (87) Chemical bonding within the three strand unit and between adjacent three strand units is primarily by H bonds. It is believed that the OH groups of hydroxyproline play an important role in this bonding by extending radially from the chains making it possible to form H bonds with the C=0 groups of adjacent strands. (67)

When collagen preparations stained with phosphotungstic acid are observed in the electron microscope, they exhibit a characteristic banding with a reappearing unit of 640 A. (73) Schmitt, Gross and Highberger (72) have found that upon treatment, purified animal collagens can be broken down into units of 2800 A. length which have the ability to reconstitute into typical banded collagen. The small-unit

form is termed "tropocollagen." It appears to be a macromolecule composed of several polypeptide chains bonded together by H bonds. The tropocollagen units are asymmetrical along their length so that orientation is a determining factor in the configuration of the reconstituted material. The 640 A banding of native collagen is now believed to be the result of a staggering of the tropocollagen units at 1/4 unit length intervals.

Collagen in situ is never found pure but always in association with other macromolecules. In fibrous collagen such as is found in the PDL, there are associated mucopolysaccharides which are bonded to the tropocollagen units in ways not yet understood. Meyer (57) feels that the bonds are probably of the polar variety. The mucopolysaccharides within the fiber contribute important properties to the composite material. Attempts to prepare surgical suture from animal collagen which has had the mucopolysaccharide fraction completely extracted have resulted in brittle and fragile products. (Personal communication, D. S. Jackson.)

At the molecular level, the collagen helix is remarkably resistant to elongational distortion. This fact has led to considerable speculation as to the fashion in which collagen fibers can subserve an elastic or stretching role such as is required of the PDL. Sicher (78) has proposed an intermediate plexus of fibers intervening between the fibers attached directly to the bone and those attached to the tooth.

He has made much of the fact that no single fiber has been traced in a continuous course from tooth to bone. To date the existence of an intermediate plexus has been well demonstrated in the continuously erupting incisors of rodents (21) and has been reported in the PDL of the whale (personal communication, D. L. Buck), but has not been demonstrated in the fully erupted teeth of man, primate or rodent. Eccles' belief that a different mechanism is involved in the fiber alignment of erupting teeth from that in fully erupted teeth seems quite appropriate. He points out that in erupting teeth the predominate fiber path is frequently parallel to the long axis of the tooth. Perhaps the best interpretation of fiber arrangement in fully erupted teeth is that of Zwarich and Quigley. (100) They report that the fibers attach to the cementum in relatively thick tufts but branch into thinner bundles toward the center of the PDL. Beyond the middle of the PDL, the fibers coalesce into another group of tufts which then insert into the bone. The inserting tufts on the bone side are smaller but more numerous than those on the tooth side. The question of whether the central branching represents a joining of fibers originating at both ends or simply an intermeshing of fibers which do not join, is as yet unresolved.

The collagenous framework of the PDL continues into the alveolar bone although it is not readily apparent with most histologic stains. The pattern of the fibrous matrix

is continuous from tooth into bone and is oriented to the prevailing stress. (87) Calcification seems to be a secondary phenomenon adapted to the demands of support and mobility. Indeed, in vitro, experiments show that nucleation, the first stage of the calcification process, occurs within the collagen fibrils themselves as a function of specific stero-chemical relations among the amino acid side chains of appropriately arranged tropocollagen moieties. (28) (In this reaction the epsilon amino groups of lycine and hydroxylycine are believed to play a key role.)

Sicher's intermediate plexus hypothesis is obviously not the only model by which one could explain the apparent elasticity of the PDL. For our present purposes it is sufficient to emphasize that the collagen fiber network of the PDL is not unique and is similar to the stroma of other dense connective tissues.

The Ground Substance of the PDL

The ground substance of the PDL is not perceived in conventional histologic preparations except as a void between the fibers. Since connective tissue ground substance generally is devoid of microscopically discernible structures, histologists before 1950 tended to treat it as a watery material with little or not support function. (5) Recent biochemical research has brought the realization that ground substance consists of highly polymerized macromolecules,

primarily muco-polysaccharides. The polysaccharide portion consists chemically of long, unbranched chains of alternating units of hexosamine and glucuronic acid with alternating 1-3 and 1-4 linkages. Mucopolysaccharides may be sulfated or unsulfated. The commonest sulfated forms are the chondroitin sulfates. The most common unsulfated form is hyaluronic acid.

One of the limitations of biochemistry as a tissue research tool is the need to obtain significant uncontaminated quantities of the material to be studied. This problem has thus far precluded direct study of thin layers like the periodontal ligament. Consequently, we must extrapolate from information available or similar materials obtainable in bulk. Meyer (57) notes that there are chemical differences between loose connective tissue as typified by dermis, and dense connective tissues as typified by tendon. Dense connective tissue has a higher fiber to ground substance ratio. Within the ground substance itself, dense connective tissue also has a higher ratio of chondroitin sulfates to hyaluronic acid. The chemical composition of PDL is probably somewhere between dermis and tendon.

We may now briefly discuss the molecular nature of some typical ground substance components. Polysaccharides are diffuse macromolecules of high molecular weight. In a sense, it is their physical properties which are of prime concern to us for it is now felt that it is their patterns

of aggregation which make them exceedingly important functional elements of connective tissue. For example, we may examine the fairly ubiquitous polysaccharide, hyaluronic acid (composed of glucosamine and glucuronic acid). The unbranched chain of alternating units is so long that were it extended the macromolecule would approach 2.5 u. In actuality the macromolecule is not extended but appears rather to occupy a spherical space or domain. It seems appropriate to compare the volumes occupied in solution by equal weights of tropocollagen and hyaluronic acid. The hyaluronic acid macromolecule has a molecular weight of around 1 million and occupies a sphere of diamter 4,000 A. (This means that within its spherical "domain" the molecule physically occupies .01 of the space.) The macromolecule of tropocollagen discussed above has a molecular weight of 345,000 and constitutes a rigid cylinder of length 2,800 A, but with a diameter of only 14 A. Thus, 3 collagen macromolecules would weigh as much as one hyaluronic acid macromolecule, but a single hyaluronic acid macromolecule occupies 25,000 times the space of the three collagen macromolecules. This illustrates the difference between the diffuse molecule of the mucopolysaccharide and the compact molecule of collagen. The domain occupied by a diffuse molecule in solution may be likened to the space occupied by a hedge or a piece of gelfoam. Such a domain has different degrees of porosity to molecules of different sizes and offers different degrees of resistance

to the passage of other molecules through it, depending more on their size and shape, than on the chemical identity of their component atoms. (74)

The observed properties of polysaccharides in ground substance can be accounted for by treating the material as a random 3 dimensional network of unit macromolecule chains. Statistically, these chains will often form regions of high chain density into which large solute molecules cannot penetrate. The volume actually occupied by the chain may include a hydration zone of fixed water of unknown size. The collagen fibers penetrate through the domain of the hyaluronic molecules so that the hyaluronic molecules not only become entangled with each other but also with the insoluable fibers. This conception makes appropriate Thurow's statement that the entire contents of the periodontal space can be viewed as a heterogenous fluid of mixed viscosities. (89, p. 75)

Resistance of a collagen fiber-hyaluronic acid pellet to compression in a centrifugal field has been shown to be considerably greater than that of purified collagen itself. (74, p. 125) This property is a reflection of the resistance of the hyaluronic acid to compression of its domain and involved the relative incompressability of the large amounts of water tied to the molecule. Similar pellets lost their elasticity and distortion-resisting capabilities when treated with hyaluronidase.

The chondroitin sulfates have considerably lower flow tendency than hyaluronic acid and are probably less water sorptive but have a more rubber like consistency than does hyaluronic acid. (9) They are found in high concentration in dense connective tissues and in cartilage. Recent autoradiographic work with S 32 (22) indicates a high sulfate incorporation rate in periodontal ligament tissue. However, the sulfate label is not unique to mucopolysaccharides since, for example, several essential amino acids also contain sulpher.

The protein portion of the mucopolysaccharide molecule has been studied only slightly thus far, but seems to be bonded to the end of the polysaccharide chain in polar fashion. In this way, at least some of the ground substance molecules are believed to terminate by direct incorporation into the collagen fibers. (57)

In young and embryonic animals, there is a higher proportion of ground substance to fibers than is the case with older animals. In newborn humans, for example, there are relatively so few fibers that it becomes very difficult to get sutures in surgical wounds to hold. (9) The low fiber ratio may also contribute to the facility of extraction or movement of newly erupted teeth. As fibers mature, the individual fibers seem to thicken. These differences in connective tissue composition may be factors in the differing characteristics of tooth movement in children and adults. It has

also been noted that ground substance material concentrations in the neighborhood of injured tissue increases early in wound healing and decreases again as a fibrous scar forms. (3)

In general, we may conclude that the periodontal ligament is a far more viscous or rubbery structure than was formerly supposed. The concept of a series of fibers running through a watery fluid must be rejected in favor of the idea of an elastic layer much less easy to distort and capable of rapid rebound towards its original state.

Extant theories of tooth movement involve a stage in which water is expressed from the ligament tissues. (89) On the basis of the chemical structure of mucopolysaccharides, such a phenomenon seems unlikely to occur in hyaluronic acid and even less likely in sulfated mucopolysaccharides at force levels as low as those developed by any orthodontic appliance.

APPENDIX D

AUTORADIOGRAPHY

Autoradiography has been defined by Boyd as the production of a two or three dimensional image or a photographic film or plate by radioactive radiation. The image may consist of a black area seen by the unaided eye or as a random distribution of grains or tracks seen under the microscope. When the image can be studied in a known spatial relation to the object containing the radioactive atoms, it can give some information about the sites of labeled molecules and about the metabolic processes in which those molecules are involved.

The autoradiographic process depends upon the fact that radioactive emissions have the property of exposing photographic emulsions. The emissions in question may be alpha, beta or gamma particles, but for biologic purposes the beta (electron) emissions are the most practical and the most important. Biological autoradiograms may be made of gross whole organisms or of macroscopic portions thereof, of tissue-cultures, or of histologic sections of tissues.

The earliest biologic autoradiograms were made by placing macrospecimens in direct physical contact with photographic plates or film. After exposure the specimens were

removed and the negatives developed. This process had limited quantitative value since the precise relationship of specimen to photograph was lost upon separation of the two.

In 1946, C. P. LeBlond and Leonard Belanger produced the first "integrated radioautographs" in which the specimen and the photographic emulsion were viewed superimposed and without separation. (7) From these early results, which were produced by coating histologic sections with melted photographic emulsion scraped from lantern slide plates, have come the present dipping and stripping techniques.

In the early years of integrated radioautography, work was mostly and almost forcibly restricted to bone and tooth work, because the labeling and substances available were all hard tissue markers. (47, p. 32) But in the early and mid fifties the work of Fitzgerald (24) and Hughes (25), the tritium label became readily available. With the development of this fairly universally usable marker, the interest of cell biologists in hard tissue studies declined considerably since most investigators understandably preferred to utilize tissues whose handling does not involve the artifact-producing decalcification process.

At present either of two autographic techniques is practical for use with histologic material. The slide may be covered by a strip of photographic emulsion or stripping film, or it may be dipped directly into a liquified photographic emulsion. The latter method, the so-called dipping

process, was utilized in the present project (40, 45, 56). In this method, a layer of emulsion covers the slide and specimen upon withdrawal from the bulk emulsion jar. This layer dries into a film which is later exposed through bombardment by the particles given off by the underlying specimen. In the present study the emulsion chosen was NTB-3, the most sensitive emulsion in the Kodak Nuclear Track Beta series.

Autoradiographic Errors

The sources of error in autoradiography may lead to either a positive or negative error (positive = excessive count or overexposure; negative = reduction of count or failure to record radioactive disentegrations which occur).

The sources of positive error may be listed as follows:

1. Errors of photographic processing.
2. Artifacts produced by chemicals within the tissue.
3. Artifacts introduced during tissue preparation.
4. Shrinkage effects.
5. Artifacts introduced by contamination or exposure of the liquid emulsion.

We will consider these in order.

1. Errors of photographic processing.--In general, nuclear track emulsions are not highly sensitive to visible light but reasonable precautions need be taken. Also,

temperatures are critical. In particular, one must avoid overheating the emulsion gel in the process of liquefying it as this will produce fog.

2. Artifacts produced by chemicals within the tissues.--The silver halides which constitute the active parts of photographic emulsions may be activated by chemical agents as well as in response to energy from electromagnetic emanations. A wide range of chemicals, particularly oxidizing agents, will produce a latent image effect. In addition, each emulsion type tends to have specific chemical activators which necessitate testing on a trial and error basis.

According to Boyd (10, p. 150), for example, NTB emulsion fogs under the influence of Cysteine and Glutathione probably due to the action of sulfhydril (SH) groups. On the other hand, it resists fogging from H_2O_2.

3. Artifacts introduced during tissue preparation.--Artifacts may be introduced during tissue preparation, particularly by the incomplete washing of the specimen to remove fixing solution. Williams (10, p. 26) reported slight fogging of NTB-3 by formalin fixed deparaffinated tissue, developed immediately after preparation. She found no effect from formalin on NTB-2 and NTB-3, but a very profound effect on G-5 (an Ilford emulsion).

4. Shrinkage effects.--Mechanical manipulation of photographic emulsions is known to cause artifacts both directly and by generating static electrical discharges. One of the

most persistent mechanical problems is the shrinkage effect which occurs as the emulsion gel loses water on hardening. This effect is much more marked with dipping techniques than it is with stripping film techniques because this type of error is inversely proportional to emulsion thickness. (Stripping film emulsion is five to eight times thicker than dipping technique emulsion.) This error is markedly increased by rapid drying techniques, a fact which was not widely realized when this project started. This effect is sharpest at the edges of specimens and is perceived as a dense peripheral exposure. The thicker the histologic section, the more marked the effect. Further, in mixed tissue sections such as ours (where the tissues themselves have a potential for uneven shrinkage due to differences in water content), this shrinkage effect will produce artifact "labeling" at transition lines between tissue types. Such effects could, for example, be found between PDL and bone, between PDL and cementum, and between dentin and pulp.

5. Artifacts introduced by contamination or exposure of the liquid emulsion.--Boyd states that the main difficulty with emulsion gel is the problem of getting it to the customer in good condition. (10, p. 10) Emulsion is extremely subject to spoilage from temperature elevations during shipping. While materials and shipping procedures have improved since his book was written in 1955, this problem is still not completely solved.

The sources of error tending to give an artificially low count in autoradiography may be listed as follows:

1. Leaching effect of chemicals;

2. Fading of the latent image;

3. Intra-tissue self absorption;

4. Failure of radioactive particles to strike a silver halide grain.

1. Leaching effect of chemicals.--Boyd notes that all histologic fixing solutions having water, alcohol, or other water soluble components will in principle remove ions and water-soluble molecules from the tissues. The same is true, of course, of other dehydrating agents. The amount of radioactivity removed depends upon relative solubility and indeed, the entire premise of autoradiography involving precursors is that the unused small precursor molecules (amino acids or nucleosides) will have become bound by synthesis into the macromolecules of protein and nucleic acids will remain behind to be counted. Controlled leaching can thus be used as an aid in tracer studies but uncontrolled leaching by indiscriminate use of histologic solutions displaces label and can lead to meaningless results.

2. Fading of the latent image.--In experiments involving long exposure times there is sometimes a problem of reduction in count due to fading of the latent image produced by emissions occurring in the exposure period. The high specific activities of currently available labeled metabolites

allows shorter exposure times and hence has tended to eliminate this problem.

3. Intra-tissue absorption.--Beta particles emanating from deep within the tissue slice may be absorbed within the tissue before reaching the overlying emulsion. If this occurs, no record of the event can be produced. The factors involved in this self-absorption phenomenon are: 1) the thickness of the slice; 2) the density of the tissue (which varies from nucleus to cytoplasm) (54); 3) the energy of the energy of the particle; 4) failure of the radioactive particle to strike an activated silver halide grain. The emulsions used in radioautography have a considerably higher ratio of silver halide to gelatine than is found in conventional photographic films. Nonetheless, it is quite possible for an electron to pass into or even through the emulsion film without contacting and consequently activating a halide grain. The probabilities of an electron causing a readable grain sensitization is dependent on: 1) the energy of the b particle; 2) the distance it has traveled through tissue before reaching the emulsion surface; 3) the precise density of halide grains in the emulsion film; 4) the angle of contact of electron with the emulsion. Considering these factors, Beiser has found that the probability of an ideal H^3 disintegration producing an exposed grain in NTB3 is .6. (54, p. 324)

The significance of the above listed sources of error

may be considered in relation to the present project. While autoradiography as a technique does lend itself to quantitative employment, the present procedure is only semi-quantitative, being limited to a comparison of relative labeling rates. For this purpose, errors which affect all observations equally will not distort results since they will not change the rank order of effect among animals. (Though the amplitudes and slopes of curves may be affected.) Further, provided sample size is sufficient, errors which are randomly distributed will not qualitatively affect the result. All the sources of error mentioned above will have equal or randomized effects if all specimens within a given run are treated identically. To make the treatments as nearly identical as possible, efforts were made to process all slides of a given run simultaneously under standardized conditions.

APPENDIX E

COMPUTATION OF FORCES DELIVERED

TABLE 1

ORIGINAL DIMENSIONS OF RUBBER BAND
(Unitek 400-154 3/8" lt. elastic)

WIDTH	THICKNESS
.876	.669
.892	.645
.909	

TABLE 2

DECAY CURVE FOR ORTHODONTIC RUBBER BAND
UNDER COMPRESSION

RUBBER	A	B
100 u	132 gms	115 gms
200	44	43
300	25	28
400	15	27
500	5	7
600	2	2

TABLE 3

DIMENSIONS OF MESIAL ROOT OF UPPER FIRST
MOLAR IN MM

SLIDE NUMBER	WIDTH	LENGTH	SURFACE AREA
103-2	.857	1.21	3.256
129-2	.787	1.21	2.990
138-2	.814	1.29	3.297
167-2	.929	1.21	3.530
			$\bar{x}$ = 3.268

(The surface area of a cylinder = πdh.
= 3.14 x width x length)

The bearing area for each root with respect to a horizontal force is equal to one half the total root surface area. (See Thurow, op. cit., pp. 73-74.) The other five roots of the first molar do not exceed the mesial root in surface area. Therefore the total bearing area of the tooth with respect to a horizontal force equals approximately $6 \times \frac{3.268}{2} = \sim 10mm^2$.

A terminal force of 5-7 grams distributed over an area of $10mm^2$ is equal to a pressure of approximately 600 grams per c m^2.

A starting force of approximately 40 grams distributed over approximately $10mm^2$ is equal to a pressure of approximately 4,000 per c m^2.

These are rough approximations, of course, but they are sufficient to demonstrate that we are dealing with heavy forces and that the pressures involved far exceed 20 grams per c m^2.

APPENDIX F

RATIONALE FOR CHOICE OF METABOLITES, INJECTION TIME POINT, AND LABEL

Choice of Metabolites

Measuring the rate of new cell formation

Cell replication requires the doubling of DNA content prior to mitosis. For this reason, measuring the overall rate of DNA synthesis is provided an excellent estimate of the rate of new cell formation. The manufacture of DNA occurs only during the so-called inter-phase. (64) In choosing a specific metabolite for tracing DNA replication, we desire to find a component material unique to DNA and not utilized in other metabolic pathways.

The DNA molecule is composed of a phosphorylated deoxyribose moiety and of four bases linked in pairs. The phosphorylated sugar moiety is not unique to DNA. The bases include the two purines, adenine and guanine, which are always linked respectfully to the two pyrimidines, cystosine and thymine. Only one of these bases, thymine, is unique to DNA since the other three are also found in RNA. (27, p. 120) Thymidine thus becomes the unambiguous choice for tracing DNA formation. (Note, while modifications of the four bases mentioned are occasionally found substituted within the DNA

helix, such modifications are relatively rare events and are hence not consequential in so gross a procedure as the present one.)

Measuring the rate of cell metabolic activity

It is presently believed that the rates of all cell metabolic activities are controlled by enzymes. The elaboration of regulating enzymes occurs at ribosomal sites in the cytoplasm on the basis of messages from the nucleus contained in the code of the m-RNA. The construction of these enzymes which are proteins further involves the activity of larger amounts of another type of RNA called sRNA. Since the elaboration of all proteins, enzymic or structural, anobolic or catabolic, involves m-RNA, sRNA and ribosomes (which also contain large amounts of RNA), the synthesis of RNA itself must precede or accompany protein synthesis. Therefore, indications of increase in RNA synthesis may properly be taken as a non-specific indicator of generalized increase in metabolic activity.

The choice of a metabolite for monitoring RNA synthesis is not so simple as is the use of thymine as an indicator of DNA synthesis. In RNA, where thymidine is not found, its place is taken by uridine. This compound, however, is not unambiguously involved in nucleic acid chemistry, being also exceedingly important in polysaccharide chemistry as a carrier of glucose, glucuronic acid, and N-acetyl glucosamine.

(85, p. 139) However, UDP-glucose is a very low molecular weight material when compared with any nucleic acid molecule. As a matter of fact, it is only one hexose sugar unit larger than UDP. Thus the compound is relatively small and relatively soluble, when compared to any of the RNAs, and we may reasonably assume that any uridine involved as UDP-glucose would be washed out of our specimen during preparation just as would unincorporated uridine. The RNA molecules, being larger, would be left behind and could then be counted.

It should be noted that both uridine and thymidine participate in the formation of nucleic acid, while assembled into their respective nucleotides. A nucleotide is a combination of base and sugar with phosphate. The most active nucleotides are the tri-phosphates. The high energy levels of the phosphorylated forms make them very difficult to obtain and very costly. Furthermore, and perhaps more important, recent work tends to indicate that the phosphorylated compounds are not transportable through the cell membrane without de-phosphorylization. Consequently in most nucleic acid work and in this experiment the nucleic acid precursors have been employed in the nucleoside form, in which the base is linked only to the appropriate sugar. Thus uracil plus ribose yields the nucleoside, uridine; thymine plus deoxyribose yields the nucleoside, thymidine.

In order for these injectables to be utilized by the animal, they must first be absorbed into the cell and then

phosphorylated, moving in a chemical direction opposite to the usual metabolic pathway. Hence we must expect that a large part of our injected metabolite will be degraded rather than used in synthesis. Nonetheless, it is quite well established that injected labeled nucleosides are indeed utilized in nucleic acid synthesis, and the procedures and rationale we have used are standard.

Measuring the rate of collagen elaboration

The monitoring of the production of any specific protein by means of monitoring the incorporation of its precursors involves serious problems since no amino acid is unique to any protein. The main amino acids found in collagen are glycine (about 32%) proline (about 13%) and hydroxyproline (about 13%). Lysine and hydroxylysine are also found as well as other amino acids in smaller quantities.

During the past several years it has been conclusively demonstrated that hydroxyproline is not incorporated into the collagen molecule directly, but is rather manufactured from proline by hydroxylation at or following its linkage into the peptide chain. (89) This disallows the use of labeled hydroxyproline in studies of collagen formation which is unfortunate since this material would otherwise be the least ambiguous indicator. The use of labeled glycine yields no particularly conclusive information on collagen formation since glycine is rather ubiguitously distributed in proteins.

Most collagen workers now consider proline to be the best available collagen tag, since proteins other than collagen do not commonly contain large amounts of this amino acid. Further, based on the work of Carniero using an experimental field almost identical with our own, it seems a fair estimate that at least 50% to 65% of the proline label observed by us is definitely incorporated into collagen. (14) Concerning the residual labeled material, there is no reason to believe that it is not distributed randomly in the same fashion in both the control and experimental animals. For these reasons, proline was chosen as the material most satisfactory for use in monitoring collagen formation.

Considerations Governing the Choice of Label

Given the choice of metabolites which we have made, two possible emittors may be used as labels. These are C14 and H3. Each has advantages and disadvantages.

The advantage of the C14 label is that it is located in the core of the molecule and is very unlikely to be displaced to a molecule of a different material which could then be misevaluated. Further, the relatively high energy level of its b emission (Mev. = .155) makes it less likely that it be overlooked due to quenching effect. This last property makes the material valuable in liquid scintillation counting.

An advantage of the H3 label is the fact that materials of higher specific activity are more easily available. Further,

the low energy level of H3 b emissions (Mev. = .027) can be turned to advantage in autoradiography by facilitating localization since the emission track is very short. The average distance traversed by a beta particle from tritium in material of the density of tissue is approximately 1 u. For this reason any activated silver grain in the emulsion can be assumed to have been energized by a metabolite whose radioactive disintegration occurred not more than 2 u distant. In practice it is customary in tritium autoradiography to consider any section over 3 u to be infinitely thick. (46, 49) In effect therefore we are measuring activity in a very small thickness which I have chosen to treat as if it were a two dimensional surface. (For further treatment of this question, see Appendix G.)

The disadvantage of the tritium label lies in the fact that the labeled atom is at the periphery of the molecule where it is subject to hydrogen exchange. (See Appendix G.) This makes it possible for the label to be lost to a molecule of another material by H-bond rearrangements leading to interpretational error. In the present experiment, it was felt that by keeping the duration of time between labeling and sacrifice to a minimum, interpretational errors due to molecular rearrangement could be rendered insignificant. Hence, the tritium label was chosen as the most satisfactory available for our purposes.

Considerations Involved in the Choice of Injection Time Point

Most autoradiographic studies of developing tissues are conducted on a so-called "fate labeling" basis. Animals are sacrificed at varying time intervals following the injection of label. In this way information is obtained on the fate of metabolic materials incorporated into the parent cells at the time of injection by tracing the path of the label attached to the precursor metabolite. The present experimant, however, was designed to provide information of a different nature. The intention was to obtain data concerning differences in incorporation rate of several metabolites at specific intervals following the induction of tooth movement. The label thus became analogous to a dye-stuff or indicator and the requirement was that it be applied to the experimental animals in a standardized fashion so that valid comparisons of the states of the animals at the instant of injection could be made. For this reason the time interval from metabolite injection to animal sacrifice was maintained constant throughout the study. In this way, temporal changes in label distribution were held similar among the animals treated with a given metabolite. The constant interval chosen between label injection and sacrifice was two hours. The reasons for this choice and an estimate of our expectations of labeling distribution two hours following injection of each metabolite need now be given.

Thymidine

Since the thymidine label was being used to measure the number of cells in DNA synthesis it was required that the interval between metabolite injection and animal sacrifice be long enough to allow incorporation of the injected material but sufficiently short so that few or none of the labeled cells will have undergone mitosis. If a large number of cells had indeed undergone mitosis, the total count would be inflated, since both of the daughter cells of each mitotic division would be labeled.

Available studies indicate that injected thymidine is very rapidly metabolized and that it will either be incorporated into DNA synthesizing cells or be metabolically degraded and excreted within thirty minutes following injection. Information on the rapidity with which mitosis follows DNA synthesis is not directly available for PDL tissues. Estimates for average mammalian cells indicate that the S or synthesizing stage lasts about 7 hours followed by a resting period (termed the G2 stage) of one to three hours. (64) Mitosis then takes place over a period of about forty minutes. Another resting period (termed the G1 stage) follows. This G1 stage is the most variable in duration but ordinarily lasts about eight hours. It is followed by the next S stage as the cycle repeats.

Thus from the point of view of guaranteeing valid DNA labeling, any time point in excess of thirty minutes would

have been satisfactory provided the time interval between injection and sacrifice were sufficiently short to preclude inflation of the cell counts by the counting of pairs of post-mitotic daughter cells. The two hour time interval seemed to fulfill these requirements quite well. Perhaps a slightly shorter period would have been even more satisfactory in terms of thymidine but this would have led to problems in terms of the proline label as will be shown.

Uridine and Proline

In the measurement of DNA synthesis one is dealing in terms of cells. For practical purposes, either a cell is duplicating DNA or it is not. A cell is either in an "on" stage (S stage) or in an "off" stage (rest of the cell cycle). The difference is therefore one of quality.

In the measurement of RNA and protein synthesis, contrarily, one is dealing with quantitative variation (since practically all cells are synthesizing RNA and protein practically all of the time). Here one is measuring differences in degree of activity. Fortunately, it can be shown that under standardized conditions the number of autoradiographically exposed silver grains is quite proportional to the number of B emissions in the underlying tissue. (46, 49) Thus, grain counting becomes an acceptable measure of incorporation of labeled proline or uridine, particularly when one makes comparative counts under standardized conditions as is

the case in this study.

In grain counting, however, a problem arises due to the superimposition of dots. This source of error can be minimized by counting with the dots spread out relatively homogeneously over the area to be counted. In order to determine the time interval best for such a state, we must briefly describe the migration of uridine and proline after incorporation within the cell.

Proline is cleared from the bloodstream very rapidly and appears intra-cellularly within minutes. All portions of the cell have now been shown to synthesize some protein, while no extra-cellular synthesis of protein has ever been demonstrated. (47) Crumley (17), Stallard (82, 83, 84), Tonna (88), and Carniero (14) all have found that heavy labeling occurs within the cytoplasm of PDL connective tissue cells within 20 to 30 minutes following injection. At one hour following injection, the bulk of the exposed grains are still located over the cell. At four hours, the supra-cellular grain count has fallen to half of the 20 minute level and substantial portions of the label are extra-cellular. By 24 to 35 hours (Stallard and Carniero) the label is mainly extra-cellular. By fourteen days the label has been turned over sufficiently to have all but disappeared from most soft tissues.

Thus from the point of view of proline grain counting, a period in excess of four hours would have been better and

probably a time point of about twenty-four hours would have been optimal. However, inordinate distortion in the thymidine portion of the experiment would have to have been accepted. At the two hour time point finally selected, we may assume that somewhat more than half of the label is intracellular.

Uridine is initially incorporated directly into the nucleus for it is there that RNAs are formed. About 5% of the RNA is messenger, 20% to 40% is sRNA, and the rest is ribosomal. The turnover is most rapid in mRNA, Mammalian mRNA is believed to have a half-life approximating four hours. (4) Ribosomal RNA has the lowest turnover.

Ribosomal RNA appears to be pre-manufactured and temporarily stored in the region of the nucleolus, which, in theory at least, would again tend to lead to grain superimpositional errors as this is a small organelle. In practice, no problem was encountered at the two hour level.

APPENDIX G

AN ESTIMATE OF THE MEASUREMENT ERROR

In order to estimate measurement error for the metabolic portion of the study, the observed effects on the opposite sides of untreated control animals were analyzed.

If there were no technique errors (in autoradiography, histologic processing or counting), and if the biological processes were equivalent contra-laterally as assumed, the difference between the contra-lateral findings within each animal should closely approximate zero. The degree to which such values for the entire experimental sample within each Run differ from zero, is a measure of the standard error of the method, or measurement error SEM $= \sqrt{\frac{d^2}{n}}$ where d = between-side difference and N = the sample size.

The values for the three Runs treated independently from each other appear in the following table:

ESTIMATED MEASUREMENT ERROR (SEM)

Calculated from Contra-lateral differences in untreated (control) animals

	N	Mesial	Distal
Run #1 thymidine (in cells/100 sq. u)	6	1.73	.35
Run #2 proline (in hundreds of counts/ 100^2 sq. u)	6	.31	.32
Run #3 uridine (in hundreds of counts/ 100^2 sq. u)	6	1.53	1.36

These differences are far from inconsequential as compared to the values for corresponding areas in treated animals (see Appendix B). A consideration of the impact of measurement error on the reliability of the data is therefore in order.

In general, it may be said that the larger the standard error of the measure, the less significant is an observation of a given magnitude in any single experimental animal. For example, with an SEM of 3.2, approximately 5% of all animals examined would show differences of 6.4 or more where no experimentally induced differences existed. This points up the importance of avoiding drawing conclusions from examination of "favorable" specimens chosen selectively from within the experimental sample. This is an ever present danger in the descriptive studies in which the dental literature abounds. To be sure, such studies are necessary and appropriate in the preliminary or hypothesis generating stage of any science but they are almost valueless in hypothesis testing when measurement errors are consequential.

When one conducts group comparisons, the larger the measurement error, the less is the precision of the technique and the more difficult it is to discern such inter-group differences as are truly present. Measurement error serves to blunt our ability to distinguish differences between groups by inflating the sum of squares and thus increasing the variance. Therefore, when between-group differences are detected

against a background of high measurement error, it may reasonably be assumed that they would be found to be present even at higher levels of significance were it possible to remove the measurement error.

Thus we draw two conclusions about our discovery of consequential measurement errors in the present experiments.

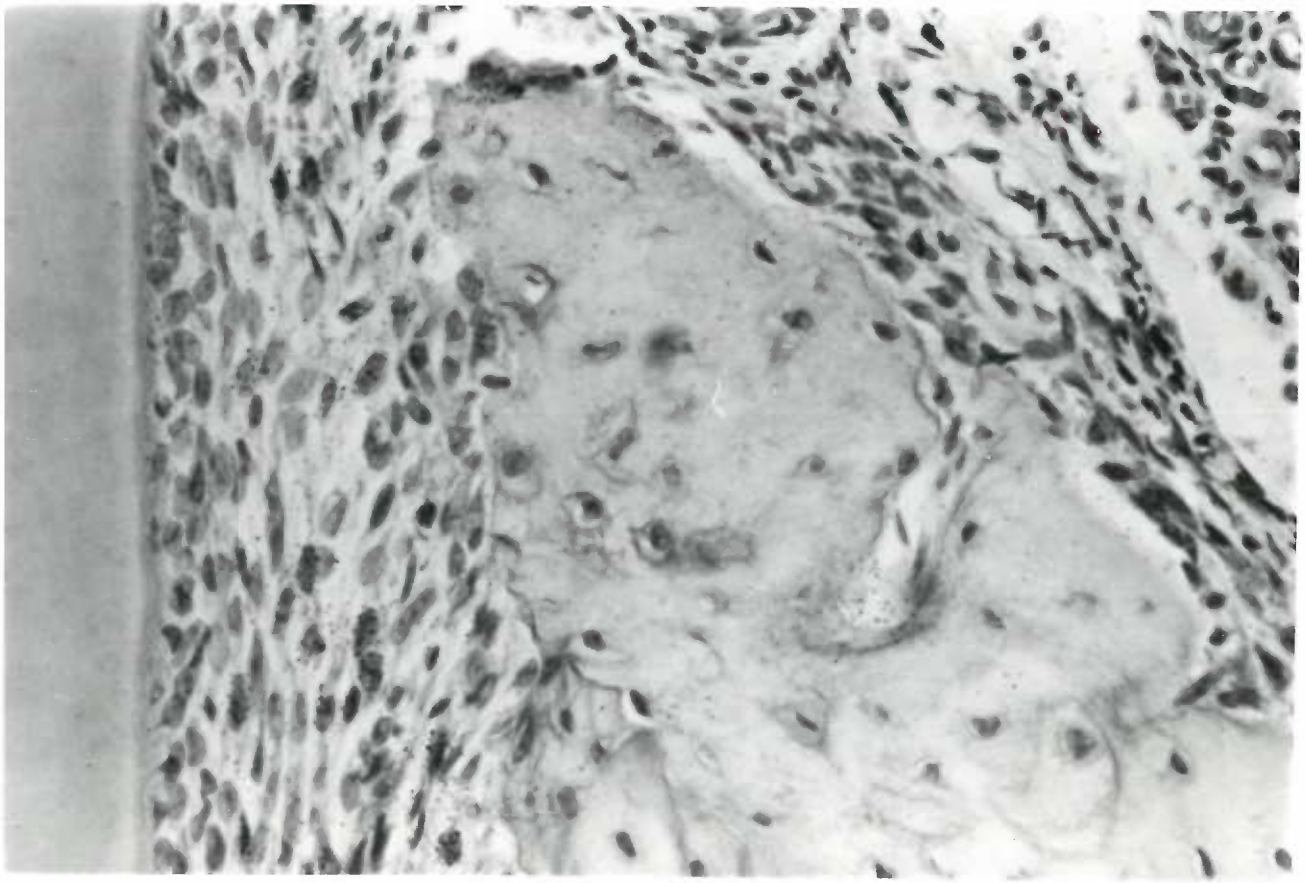
1. It renders unreliable any statements based on single individuals selectively sub-sampled and

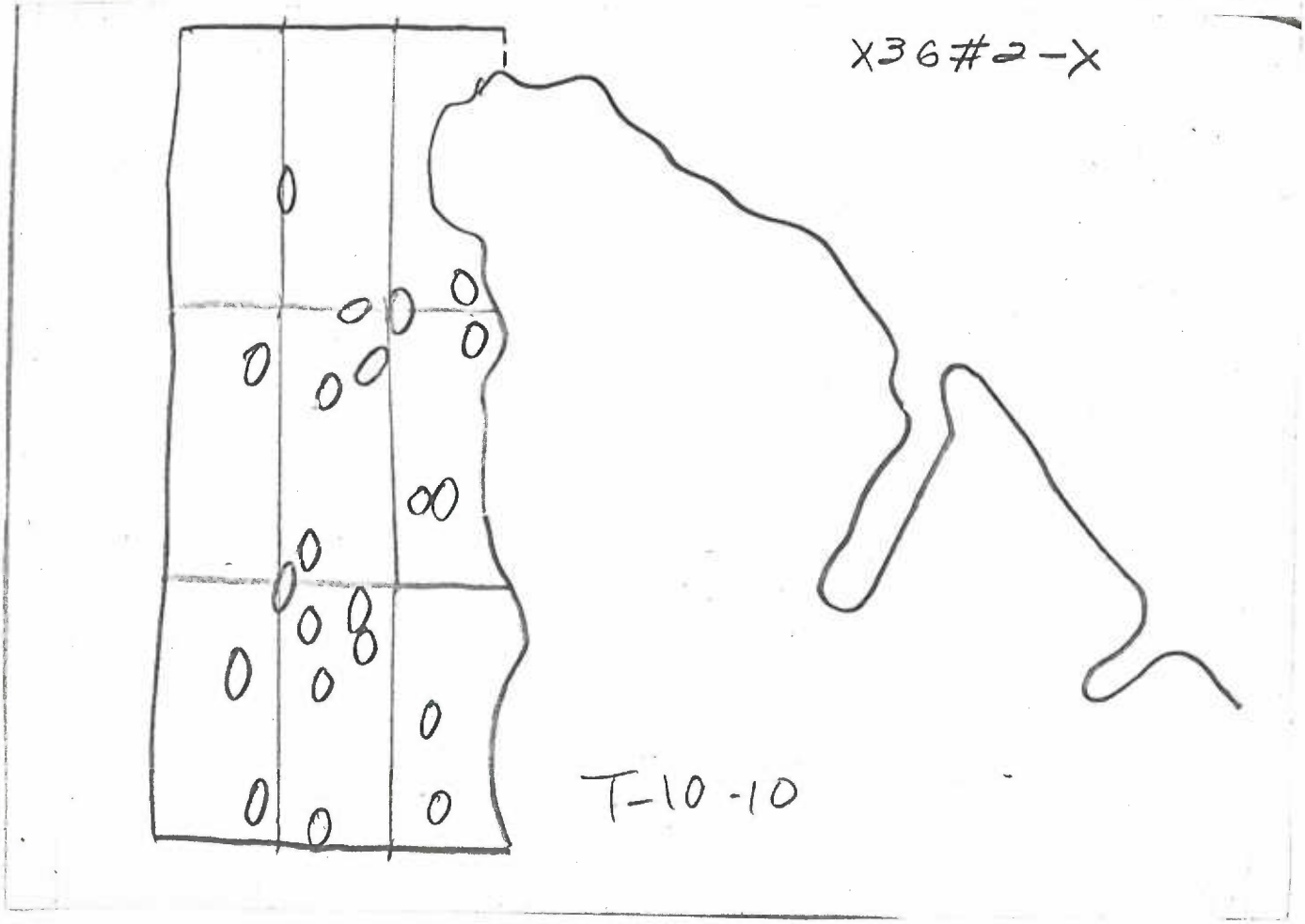
2. The finding of significant group differences may be taken as significant at the indicated level or higher.

It should also be noted that, while we have been talking of randomized measurement error, there does exist the possibility of a systematic measurement error not discernable by the present analysis. Fortunately a systematic error which affected both sides of the animal equally would not be of great concern to us since such an error would have no consequence more serious than shifting the base line. Such a shift would not be important since our study is not really concerned with absolute quantities but rather with differences. A systematic error affecting only one side of the animal would indeed be alarming, but there is no reason to believe the existence of such a phenomenon and every reason to doubt it.

APPENDIX H

Representative Illustrations

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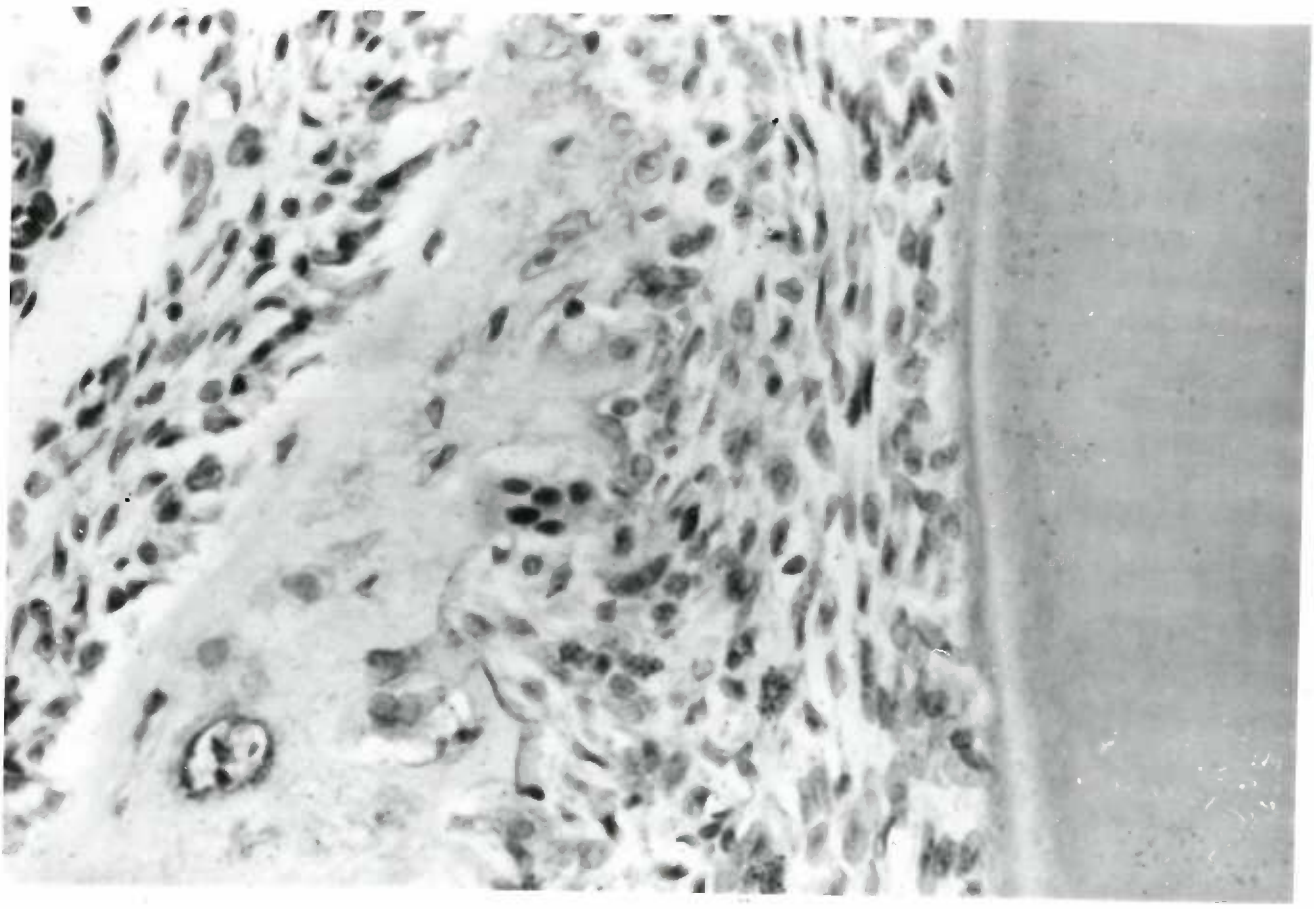
T-10-10

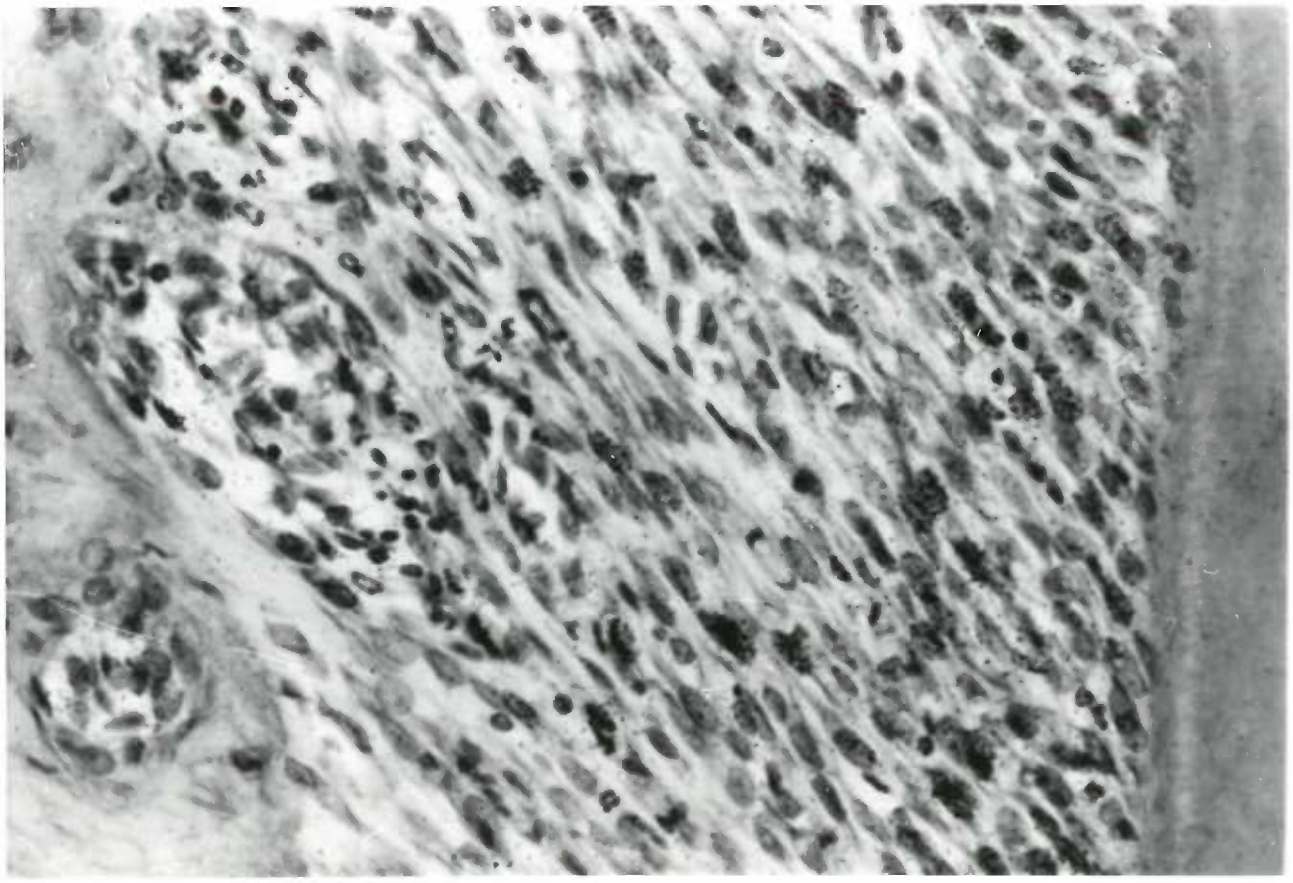
THYMIDINE Experimental 48 hours "pressure control" side

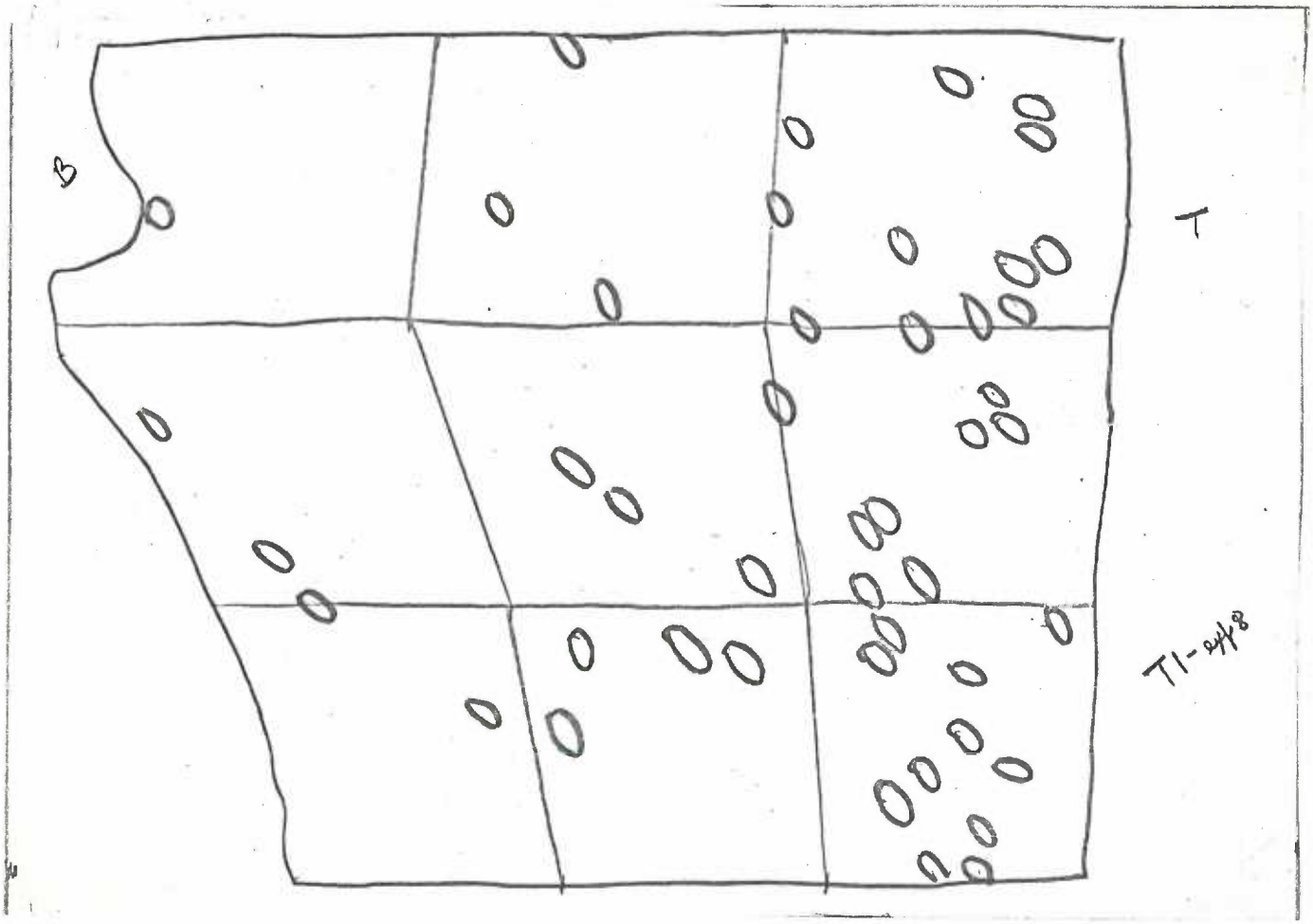


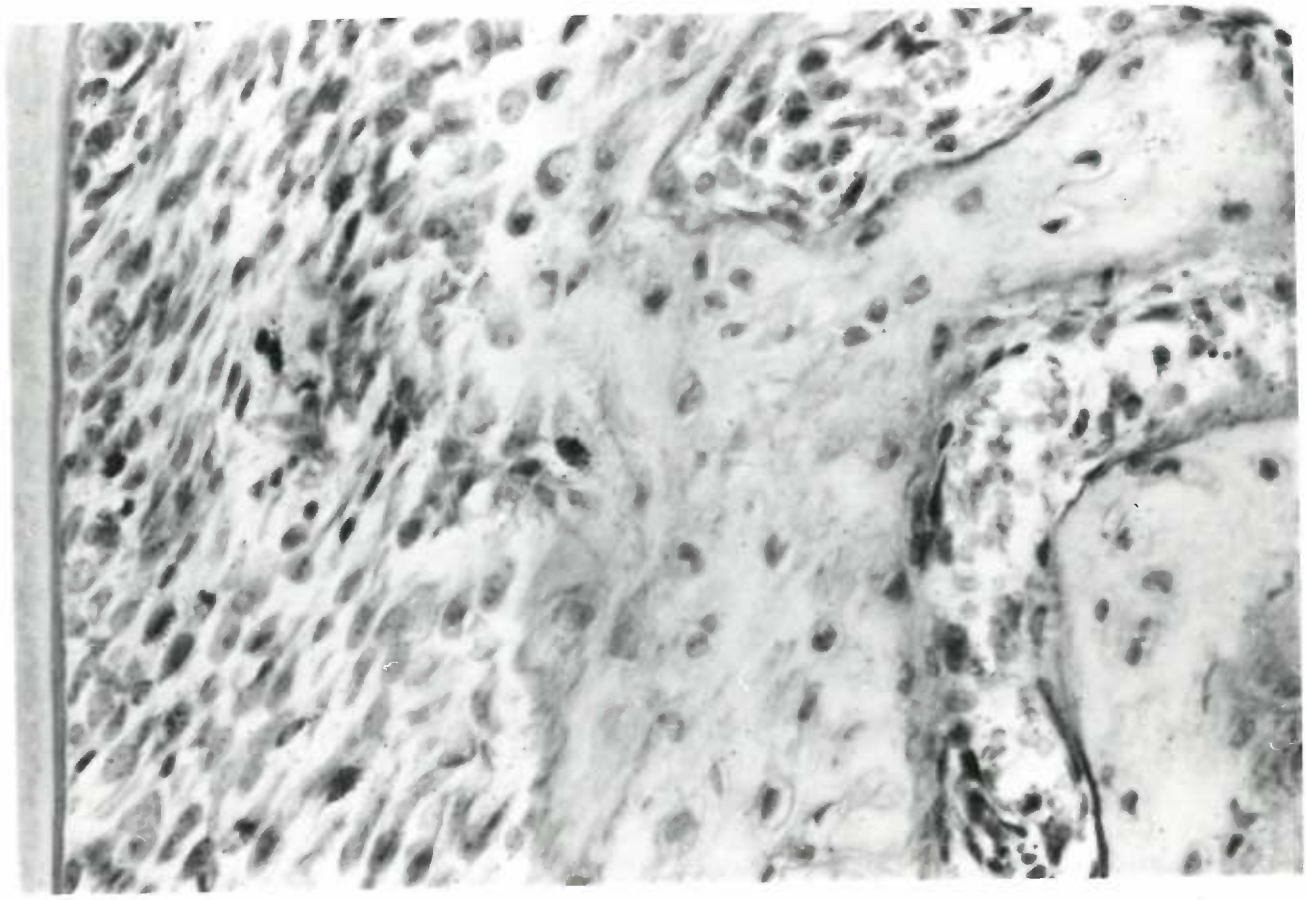
X48#1-C

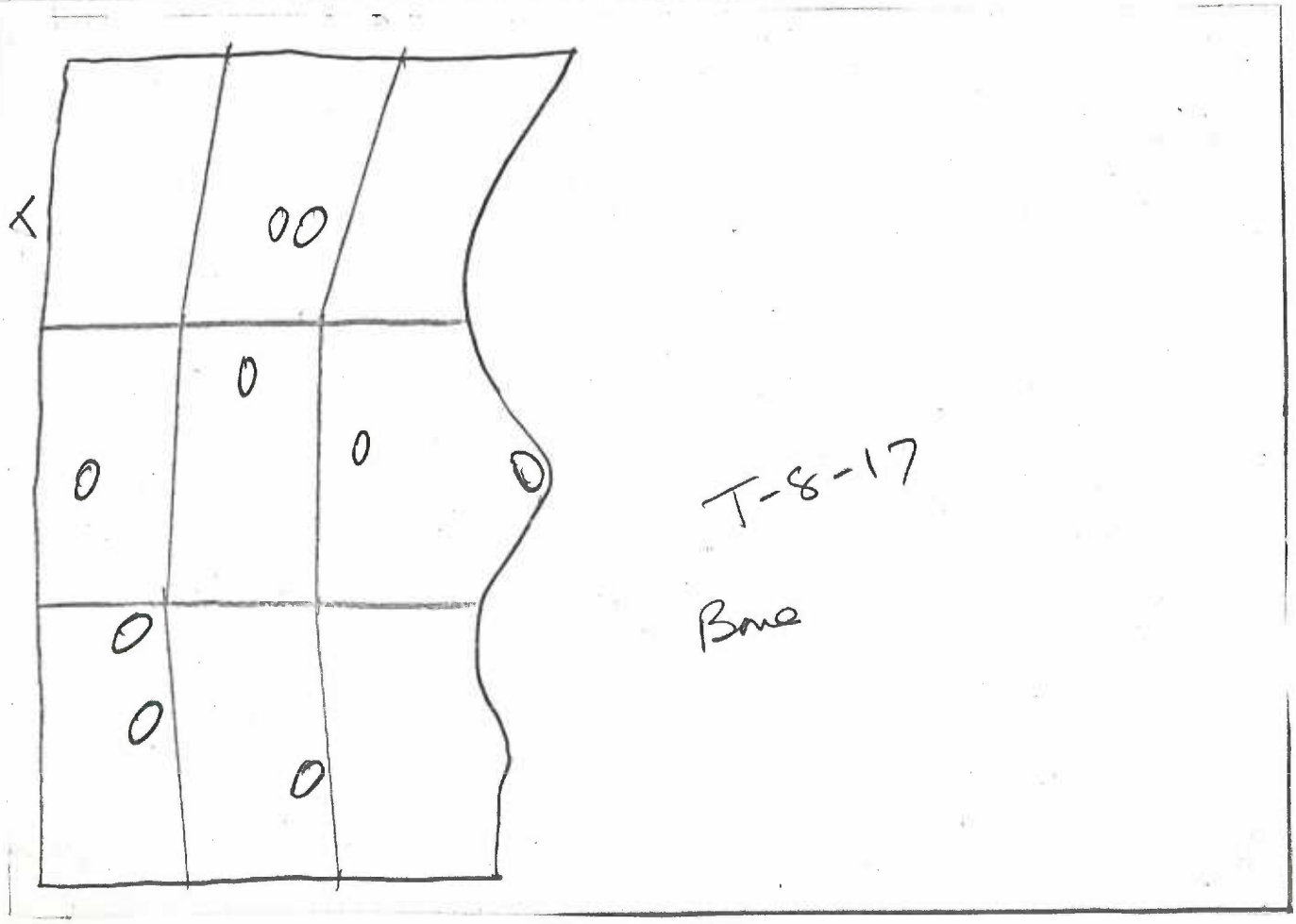
T-13-11

THYMIDINE Experimental 24 hours "tension" side





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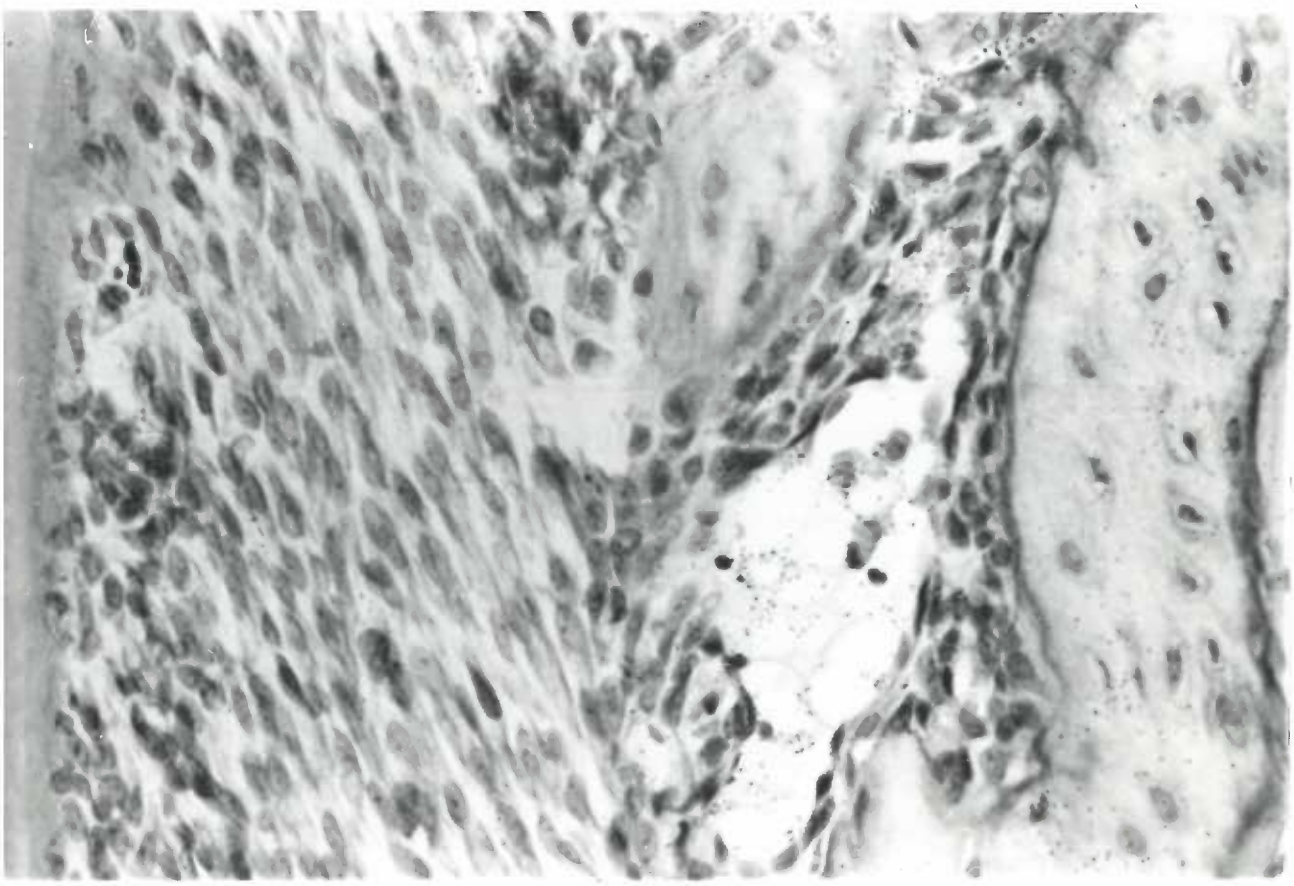
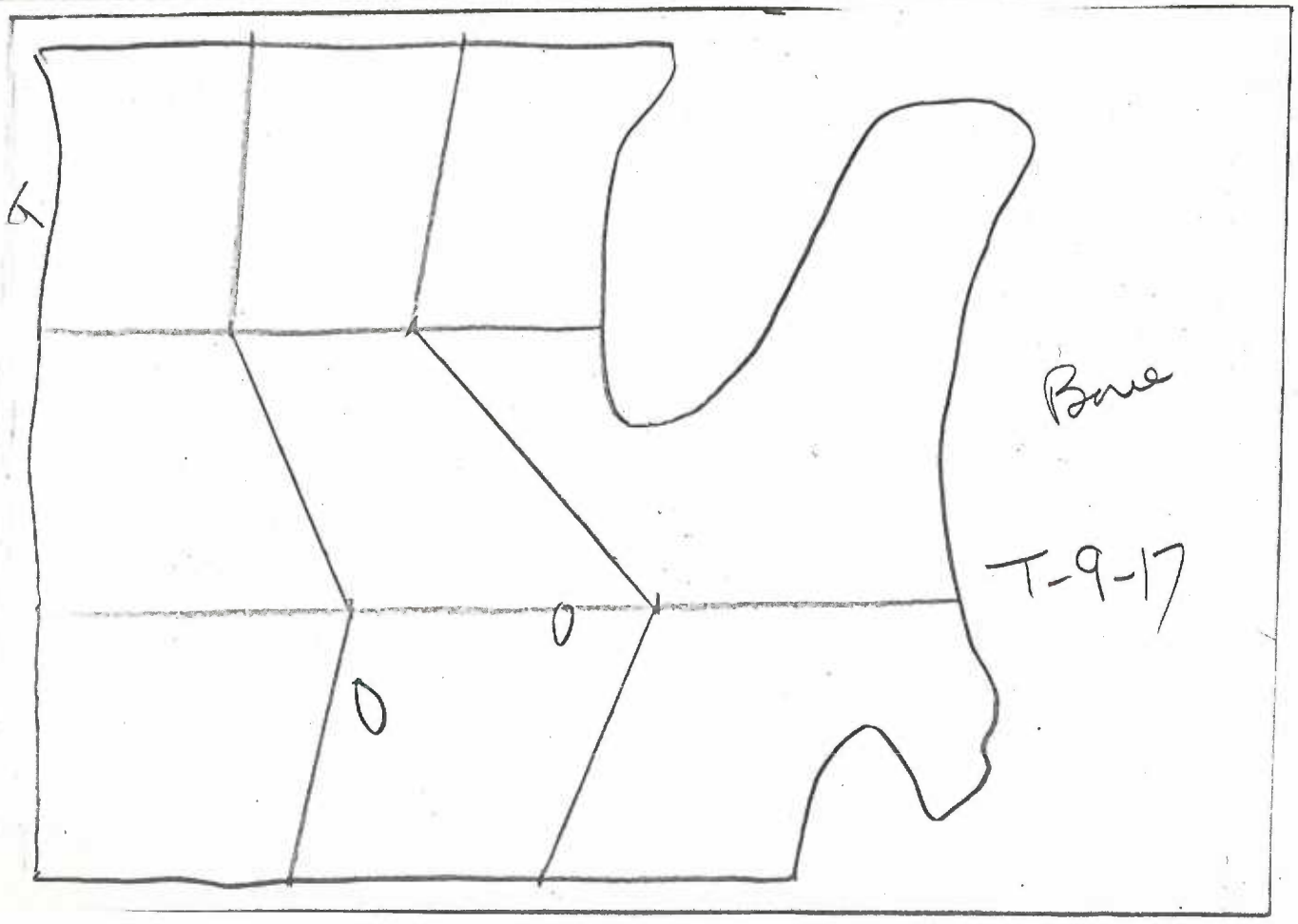
T-8-17
Bma

THYMIDINE control 72 hours "tension" analog (no T. M.)





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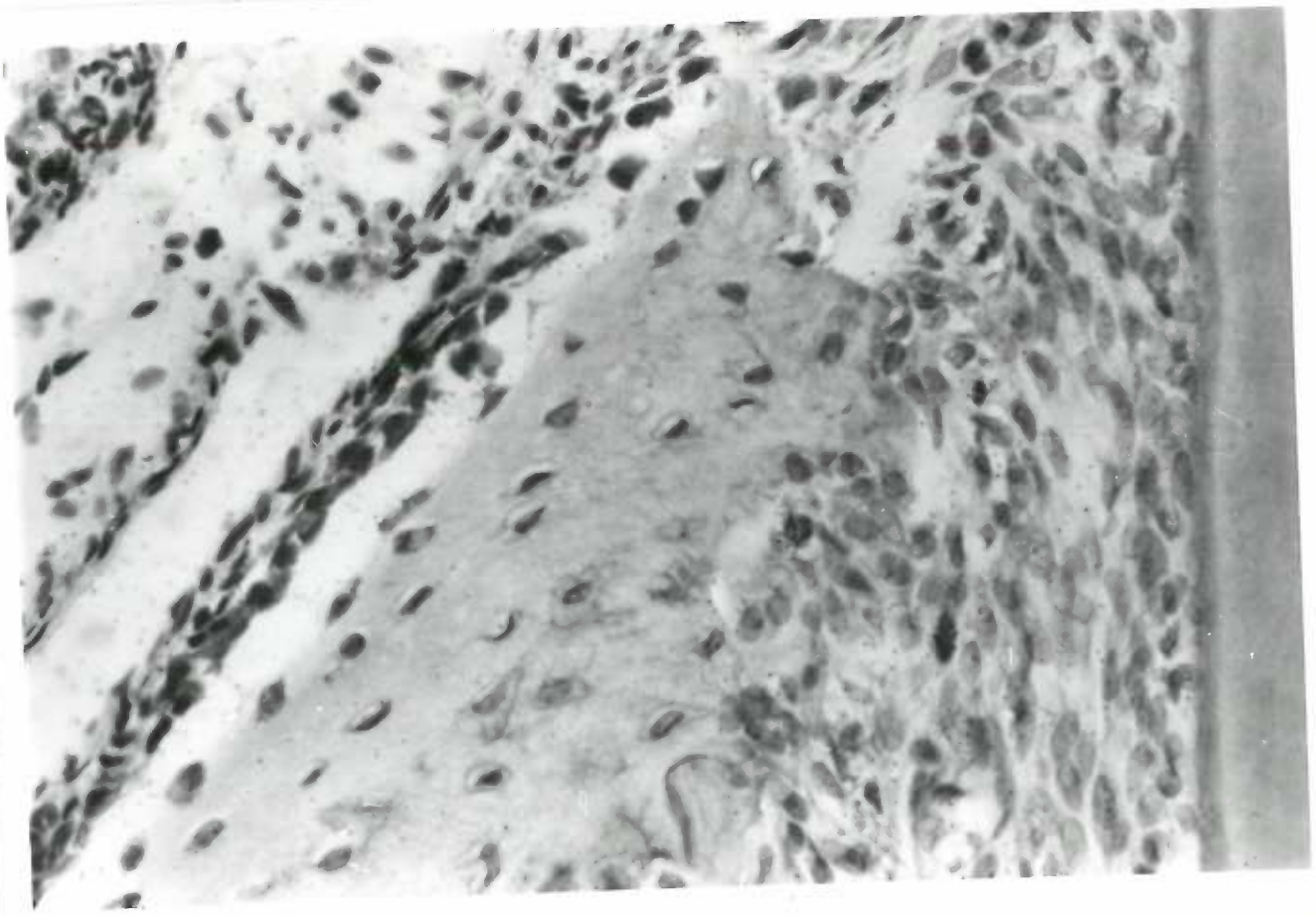
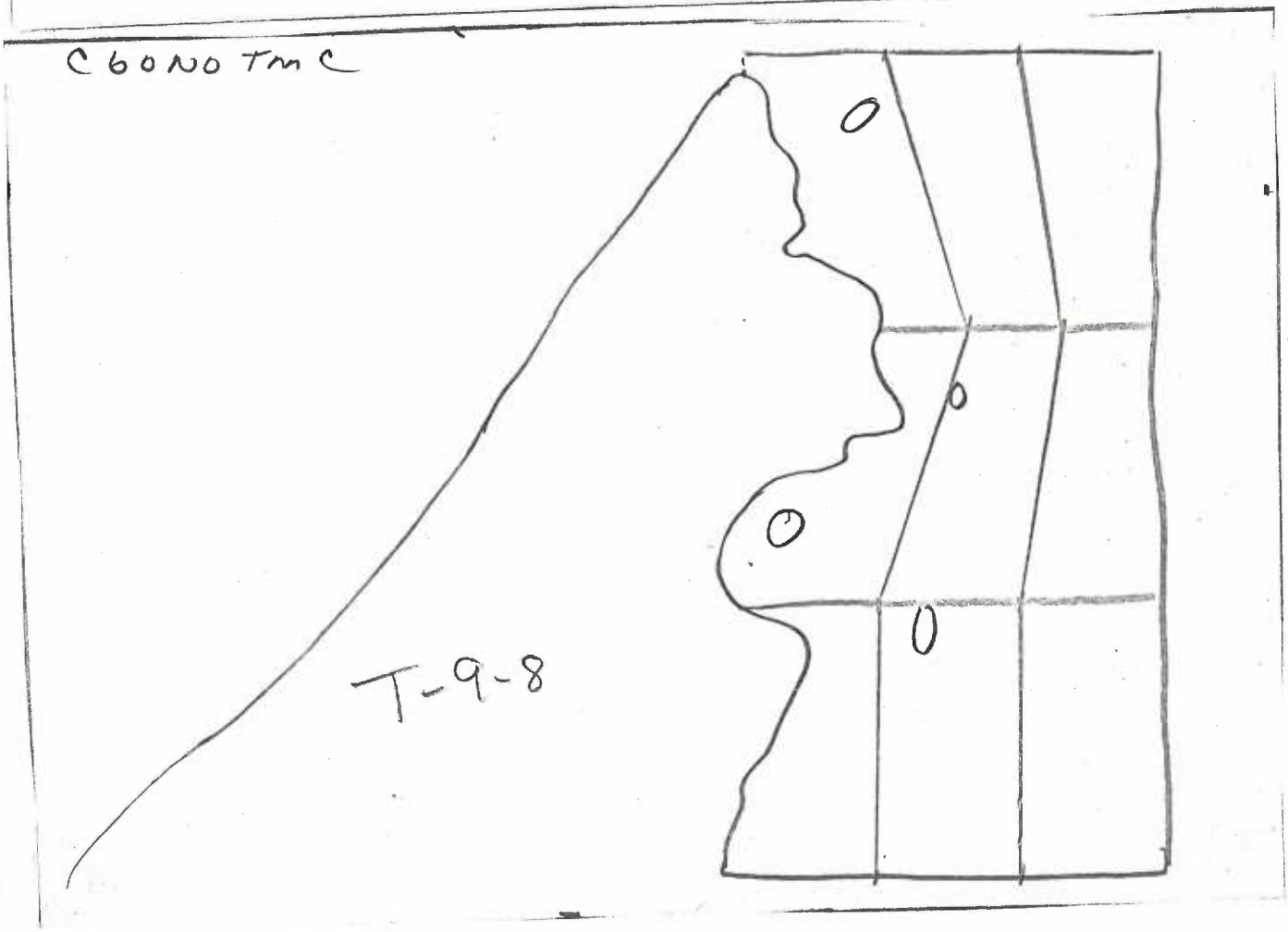
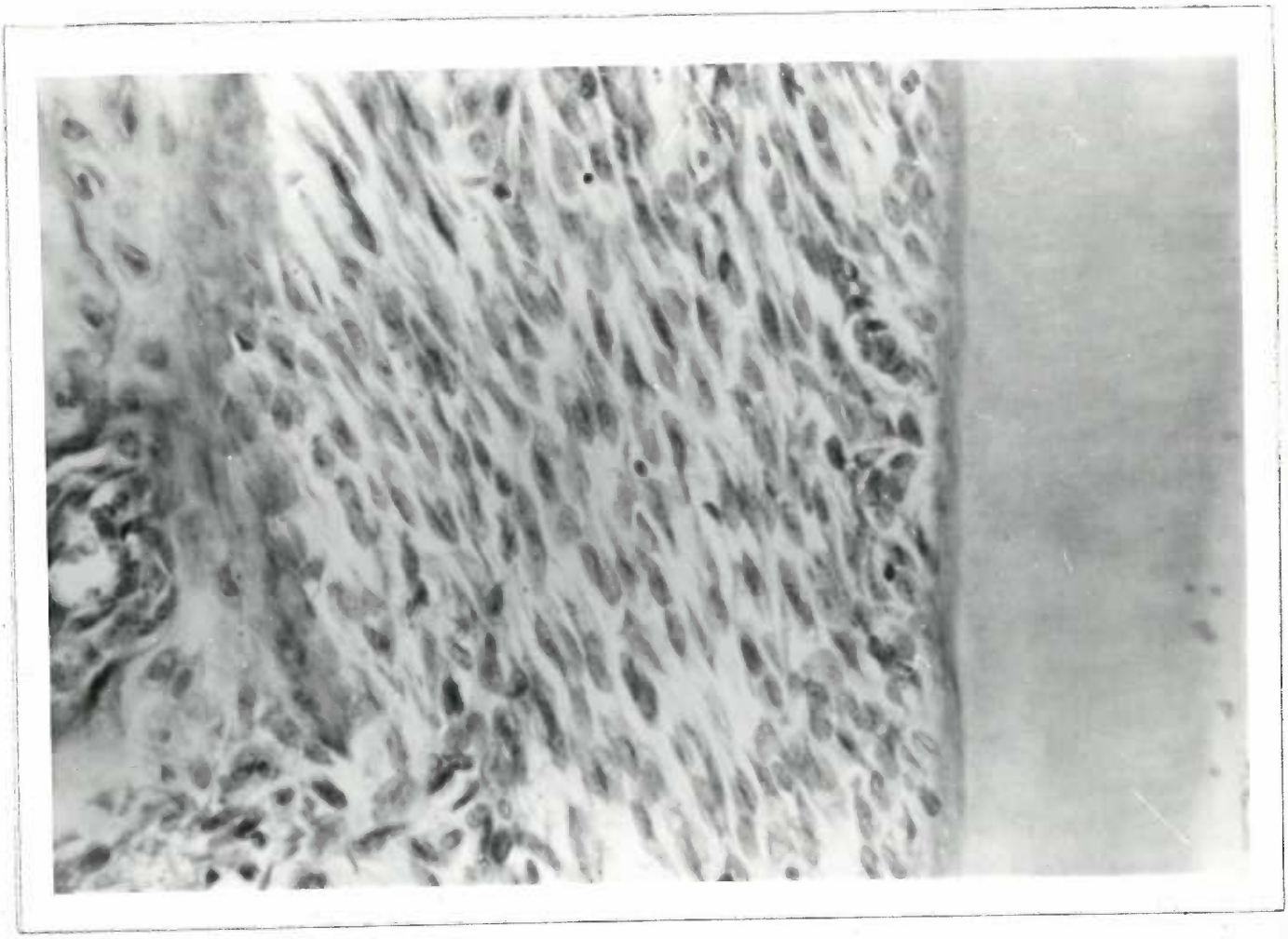
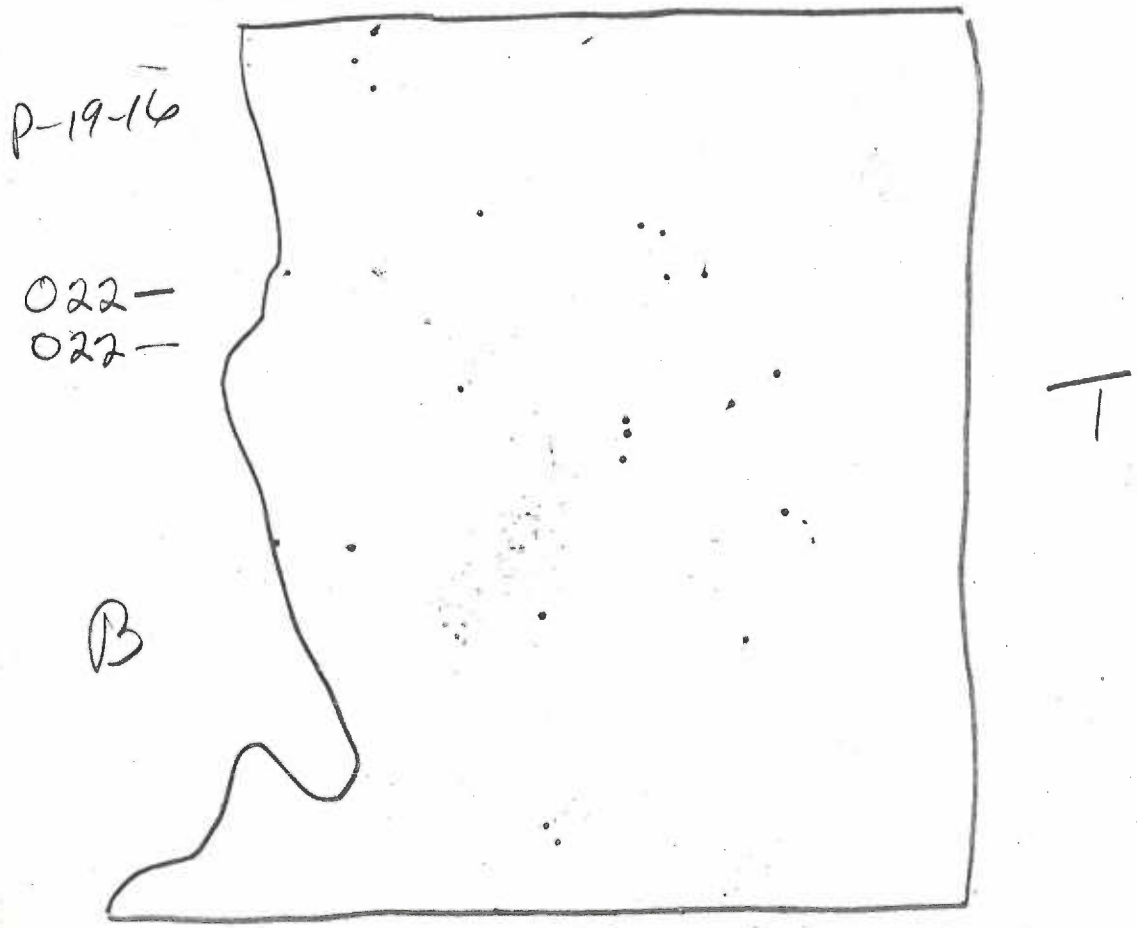


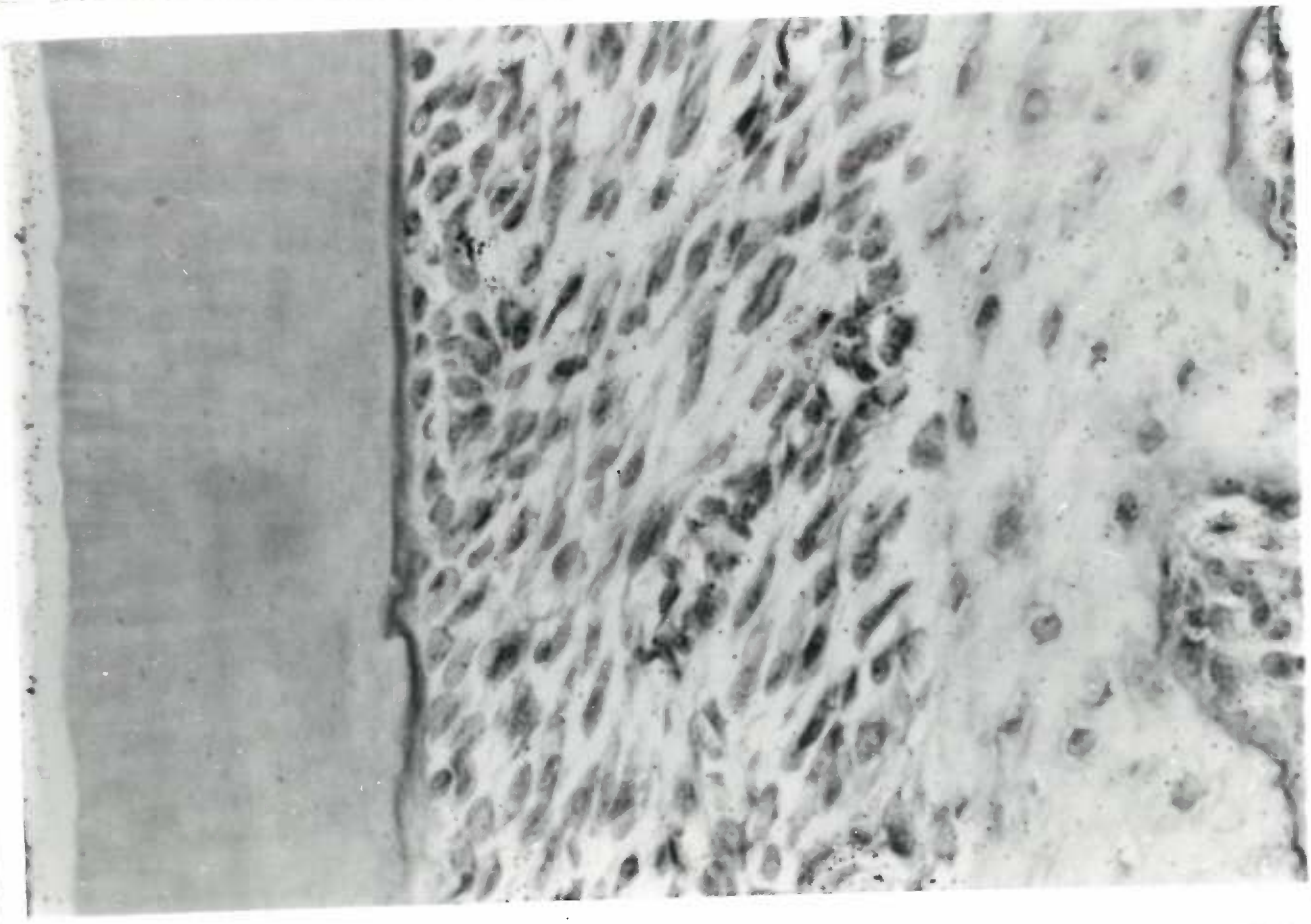


PROLINE Experimental 48 hours "tension" side





PROLINE Experimental 12 hours "tension" side



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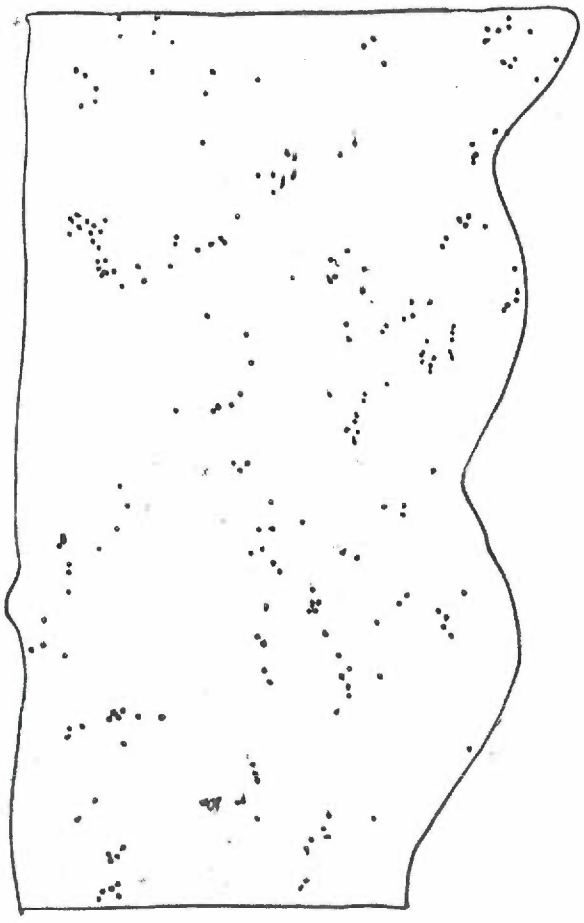
0223—

T



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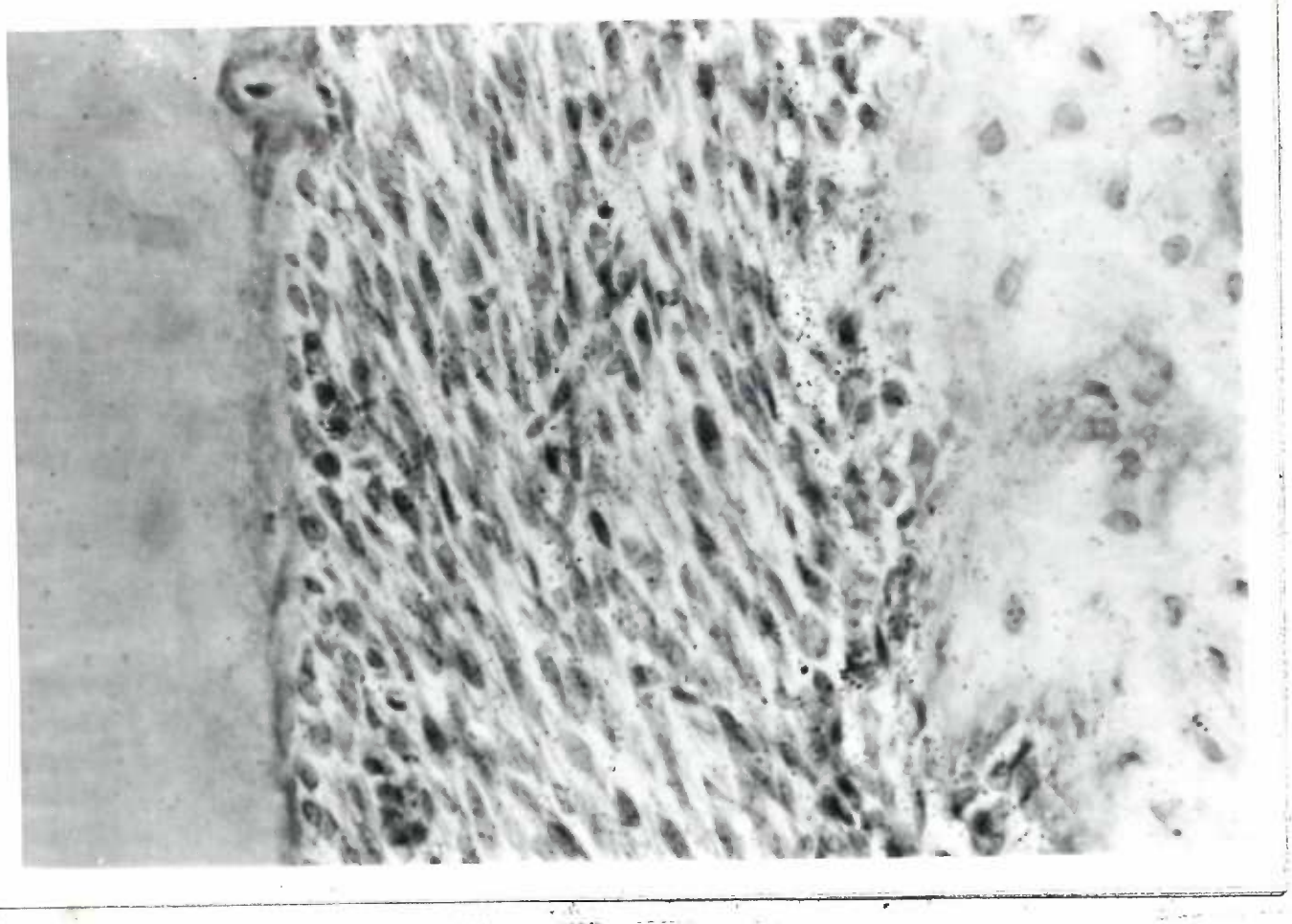
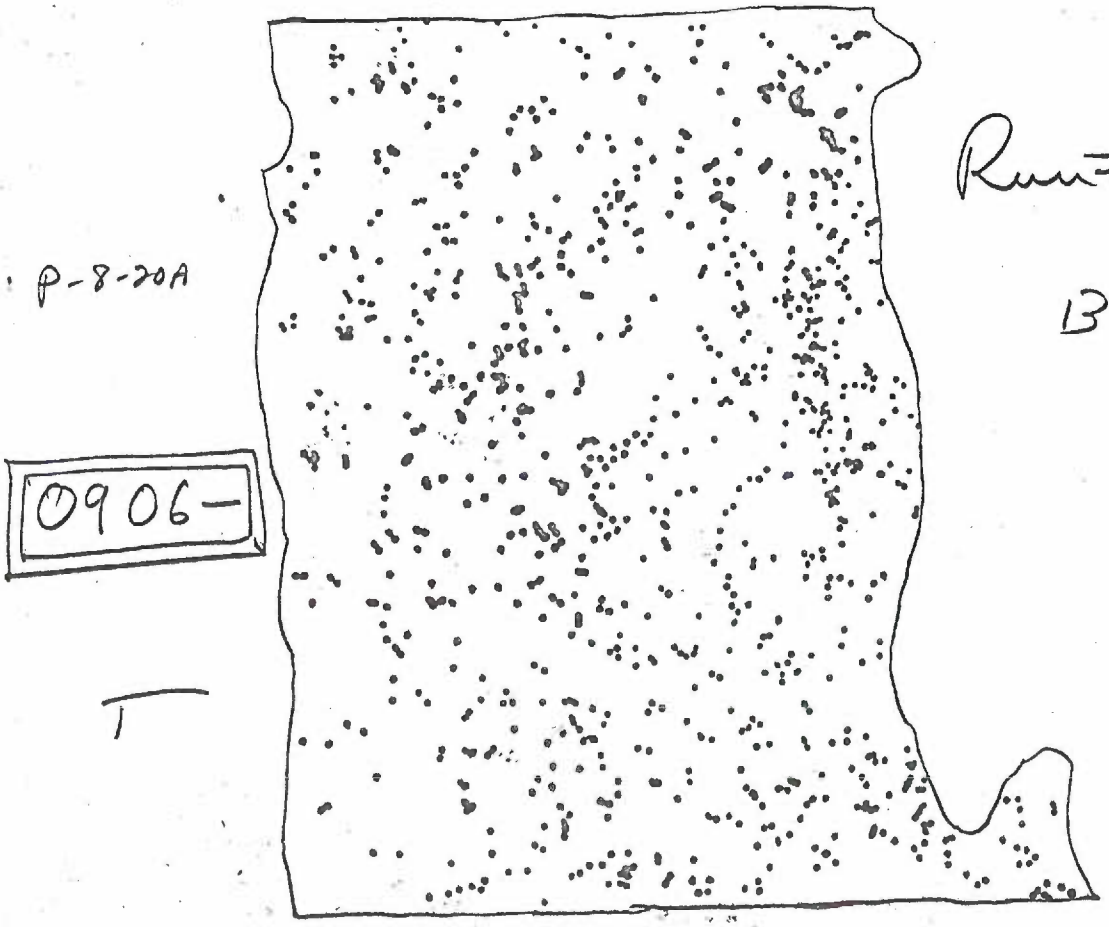
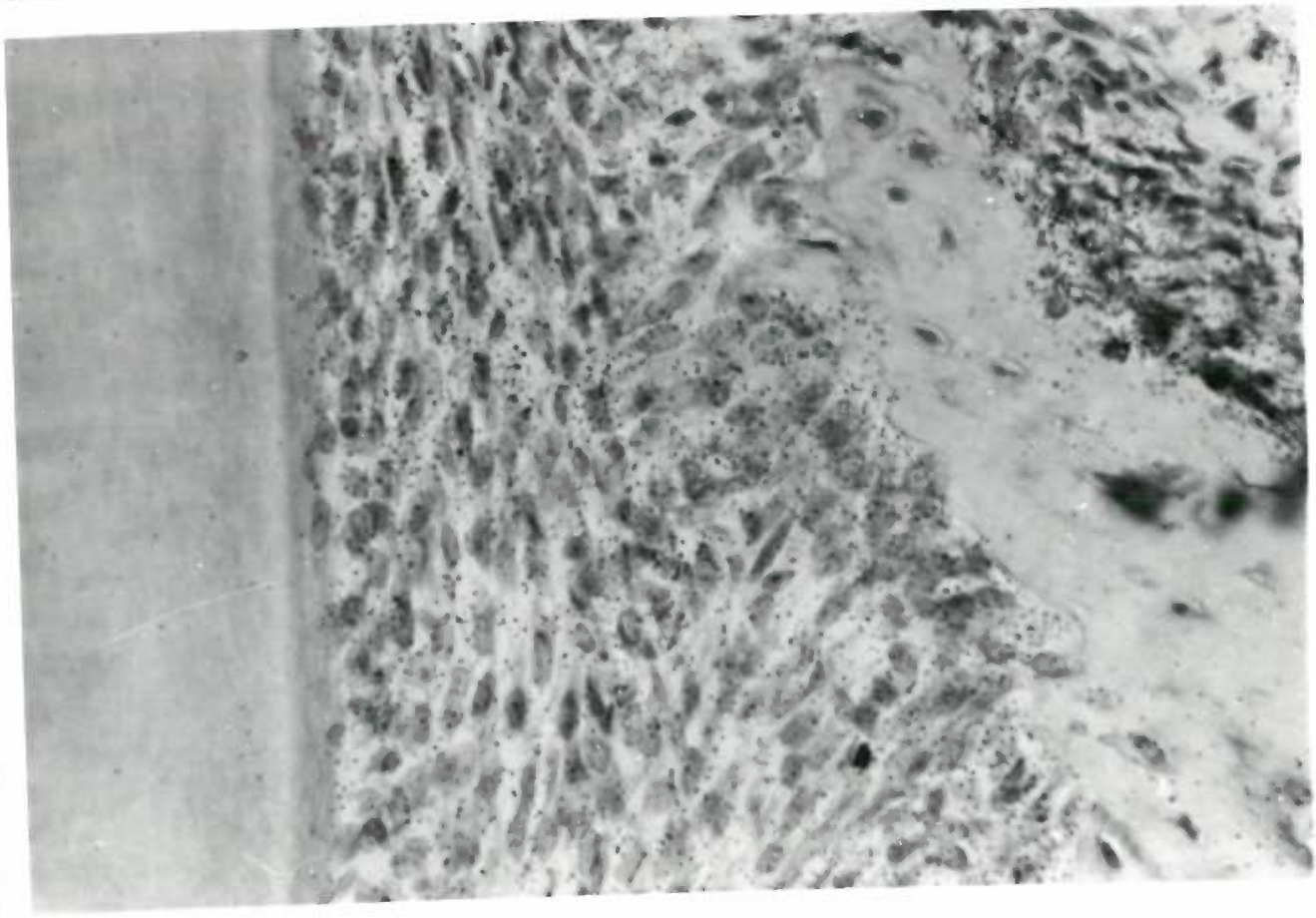
PROLINE Experimental 24 hours "tension control" side





PROLINE Control 60 hours "pressure" analog (no T. M.)



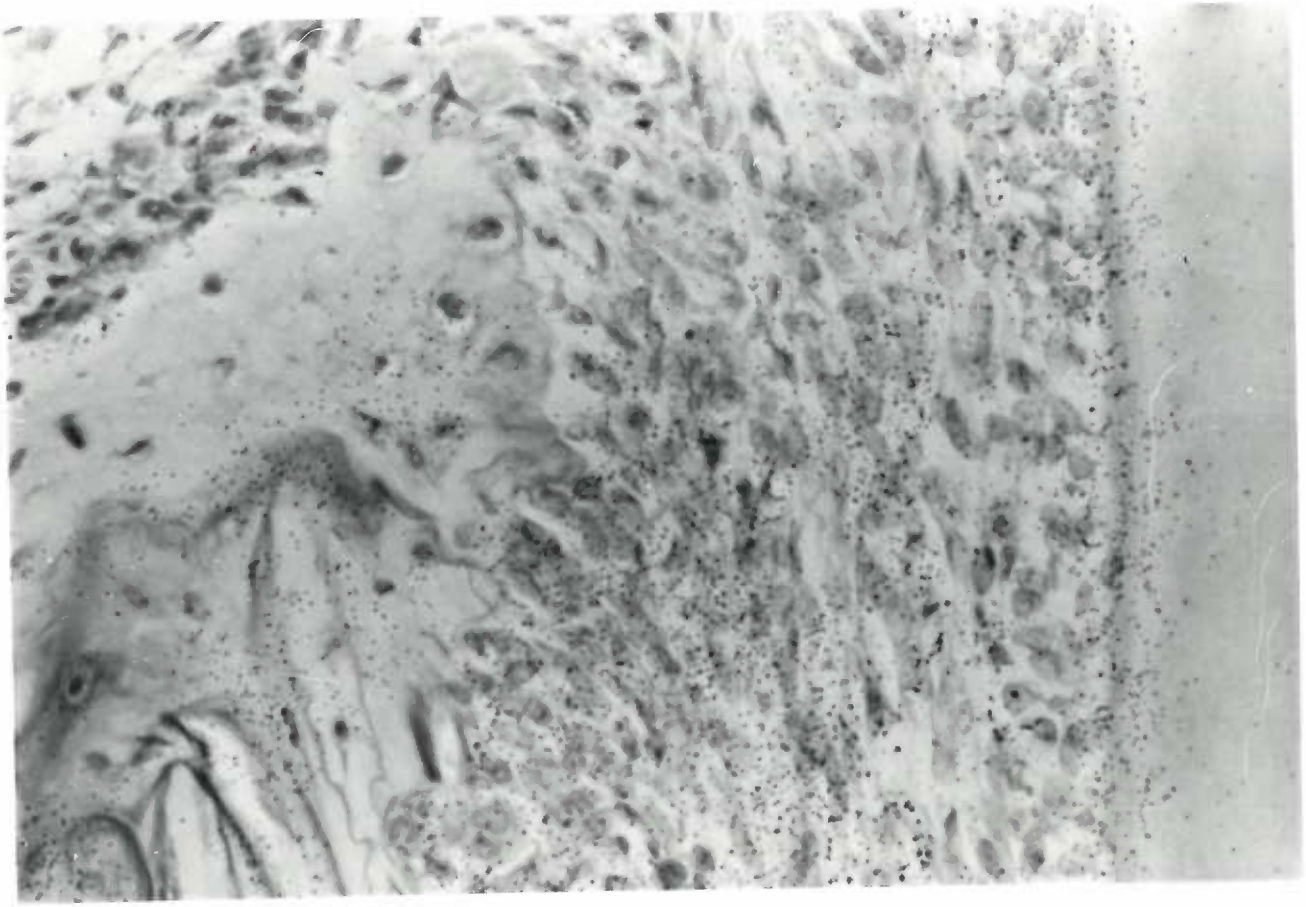
P-16-9

T

02260—

B

URIDINE Experimental 42 hours "pressure" side



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B

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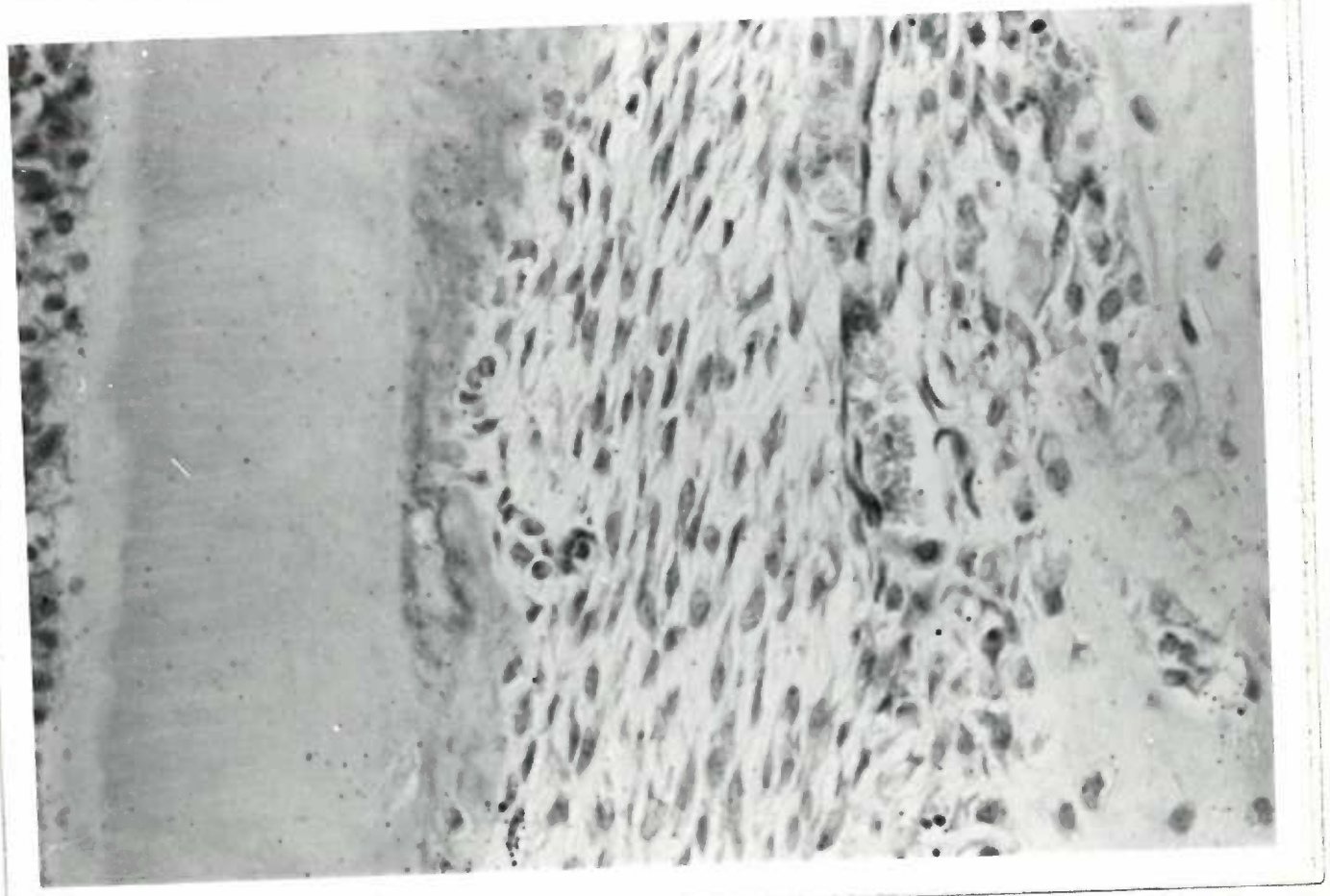
URIDINE Background Control (no label) "tension" side



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098—

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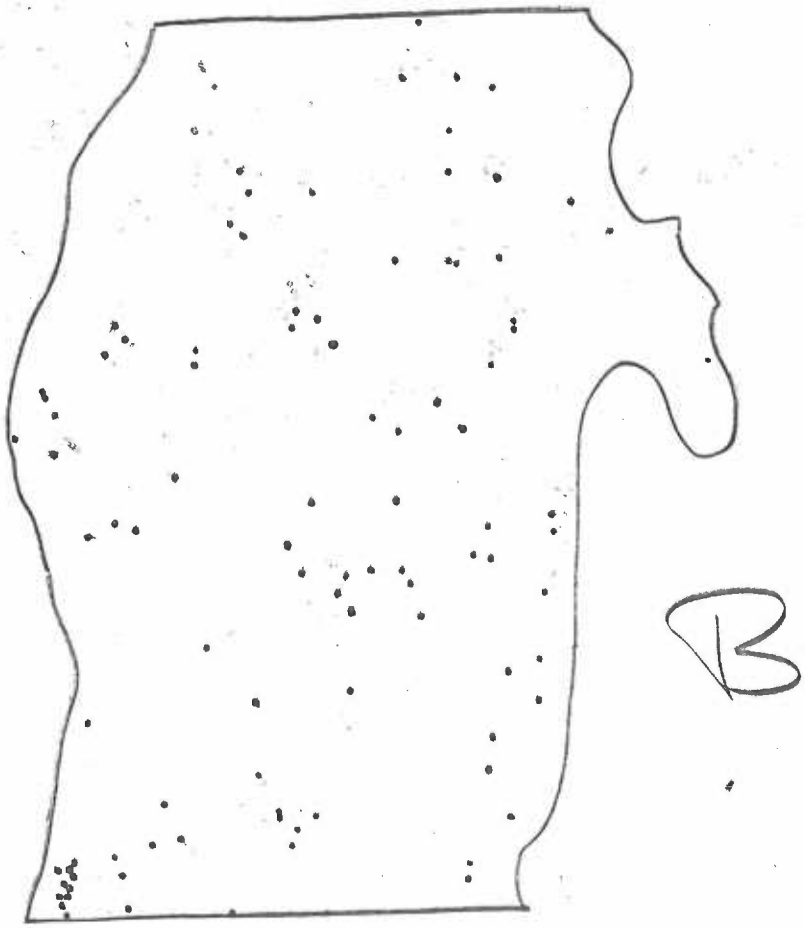


B

Figure 1

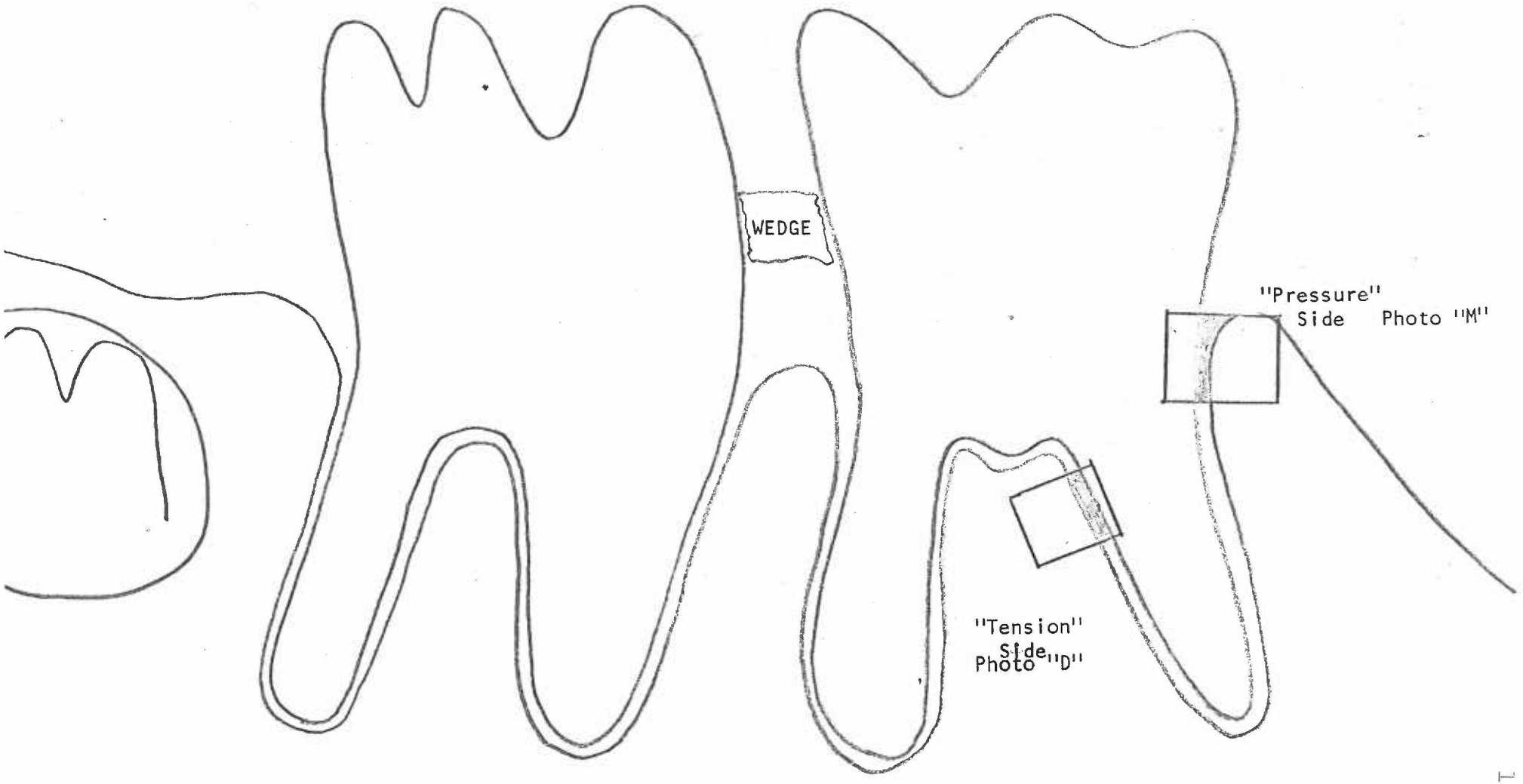
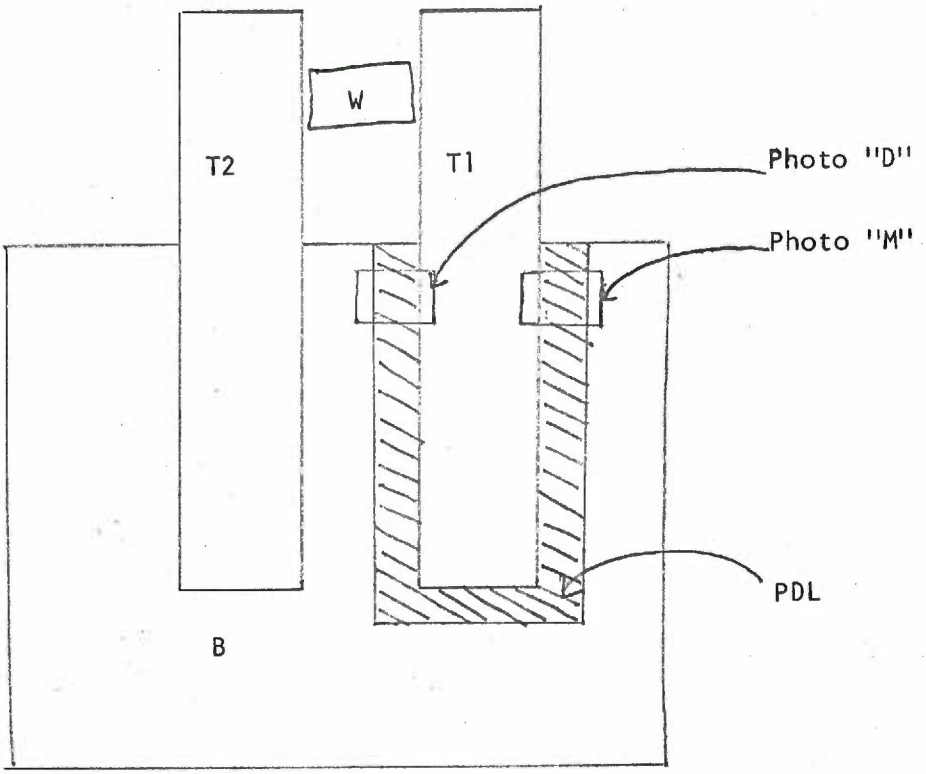


Figure 2. Schematic Representation of the Experimental Field



This drawing is simplified by the omission of details not salient to the present experiment - -

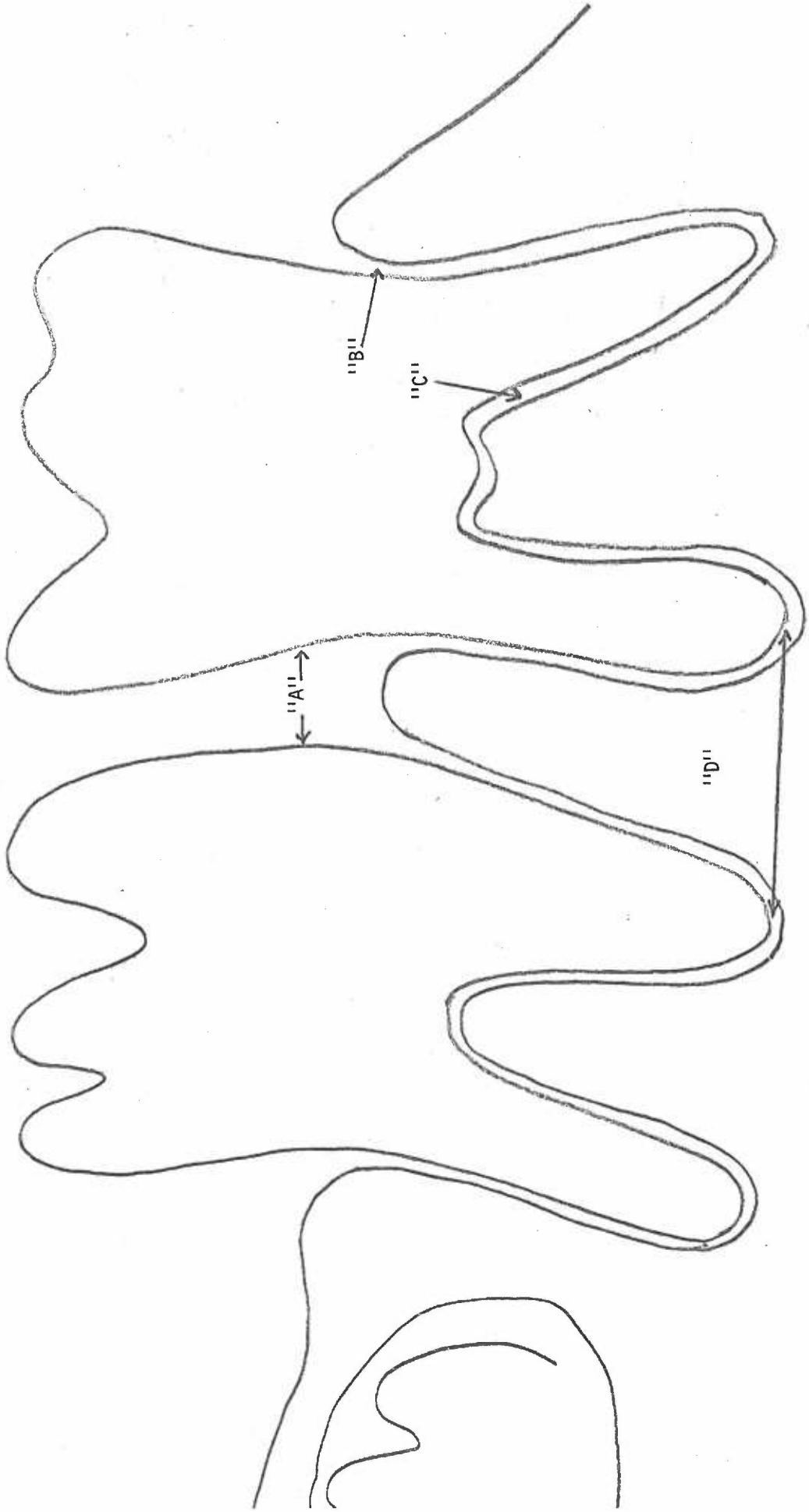


Figure 3

ABSTRACT

ABSTRACT

The present study has two parts.

The first part represents an attempt to measure changes in several parameters of cellular activity incident to tooth movement. Three independent samples, each consisting of 33 young Sprague-Dawley rats, were used to measure

1. Changes in rate of cell replication in the PDL,

2. Changes in general metabolic activity rate in the PDL, and

3. Changes in rate of collagen synthesis in the PDL,

during the first seventy-two hours following the induction of tooth movement by the unilateral placement of an elastic wedge between the maxillary first and second molars. Each run included six control animals in which no elastic had been placed, making possible three-way comparisons of experimentally treated teeth, untreated contra-lateral teeth in experimental animals, and completely untreated analogous teeth of control animals. Evaluations were made autoradiographically by grain counts and cell counts.

With regard to changes in rate of cell replication as measured by the incorporation of H3 thymidine, the following

results were noted.

On the "pressure" side of the root, contrary to expectations, activity in the PDL of treated teeth was significantly greater than that in analogous PDL areas of untreated teeth in the same experimental animals. Treated areas in experimental animals gave higher values than did similar areas in control animals. On the "tension" side of the root, both treated and untreated teeth in experimental animals showed increases in cell replication rate above the base line established from control animals, but no significant differences were found between treated and untreated teeth in the same experimental animal.

With regard to changes in general metabolic activity rate in the PDL as measured by change in rate of incorporation of H3 uridine, chance bilateral variation within the control animals was sufficiently great as to render the results inconclusive.

With regard to changes in collagen synthesis, as measured by change in the incorporation rate of H3 proline, all tested areas in the PDL of the experimental animals showed statistically significant declines in synthesis when compared to analogous areas in control animals. No significant differences were detectable between treated and untreated areas within the same experimental animal.

In the second part of the study, data from 90 animals

of the three Runs were pooled in an attempt to identify dimensional changes in the periodontal tissues consequent to the introduction of an interproximal force.

It was found that the crown of the first molar moved an average of 200 u following placement of the elastic but that no discernible change in the distance between the apices of the approximating roots of the first and second molars was produced. It was therefore concluded that an almost pure tipping force had been applied, yielding an axis of rotation rotated at or very close to the apex of the distal root of the first molar.

The reduction of width of the PDL on the "pressure" side of the studies first molar root as a result of force application averaged only 9 u or about 10% of the PDL width. Since the same force caused a change in crown position of 200 u (twenty times the change in PDL width) one is forced to the inescapable conclusion, for the present model at least, that deflection of the investing bone has occurred.

These results strongly imply that bone deflection can be produced by forces lower than those required to produce significant width reduction in the periodontal ligament. It is felt that this hypothesis bears testing in animals whose dentition is more similar to humans than is that of the rat.

IX

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