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ABSTRACT

The extensive role that prostaglandins play in the cellular metabolism of living organisms is just beginning to be elucidated. Nearly all organ systems are in some way affected by prostaglandins. Bone resorptive osteoclastic activity has been shown to have a connection to the biologic activity of this lipid family. The connection is poorly understood but has nevertheless led to clinical ramifications. Of particular interest, currently in orthodontic literature, is the effect that prostaglandins have on the rate and quantity of tooth movement. High local concentrations of prostaglandins can result in an increased rate and quantity of tooth movement while prostaglandin inhibitors can have the opposite effect.

Utilizing 42 Hartley guinea pigs, two experimental groups and one control group were established to examine the influence of therapeutic doses of aspirin and acetaminophen, both prostaglandin synthesis inhibitors, on clinical and histologic aspects of orthodontic tooth movement. Clinical and histologic results revealed no statistical differences among the controls and either of the experimental groups.

Given the influence that prostaglandins have on tooth movement, it seems reasonable to recommend against the use of prostaglandin inhibitors for the relief of orthodontic discomfort. However, this study did not produce evidence to contraindicate the use of prostaglandin inhibitors, by orthodontic patients, on the possibility that tooth movement will be significantly slowed down by consumption of the drugs. These, (1) -R-

THE INFLUENCE OF ACETYL SALICYLIC ACID
(aspirin) AND ACETAMINOPHEN ON CLINICAL AND
HISTOLOGIC ASPECTS OF ORTHODONTIC TOOTH MOVEMENT

by
Charles E. Bedell

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Orthodontics
in the Graduate College of
The University of Iowa

May 1988

Thesis supervisor: Professor Charles R. Kremenak



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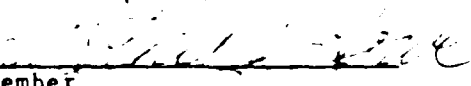
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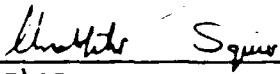
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has been approved by the Examining Committee
for the thesis requirement for the Master of
Science degree in Orthodontics at the May 1988
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Thesis committee:


Thesis supervisor


Member


Member


Member

Dedicated to

My wife Patti

and to all the men and women
of the United States Air Force

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INTRODUCTION

The extensive role that prostaglandins play in the cellular metabolism of living organisms is just beginning to be elucidated. Due to the ubiquitous nature of prostaglandins nearly all organ systems are, in some way, affected by them. It is no surprise that bone resorptive osteoclastic activity has some connection to the biologic activity of prostaglandins. This connection is poorly understood but has nevertheless led to possible clinical ramifications.

Several investigators have utilized the relationship between osteoclast activity and prostaglandins to affect a change in the rate of tooth movement associated with orthodontic mechanics. Locally high concentrations of prostaglandins appear to accelerate orthodontic tooth movement through increased activity of osteoclasts. Conversely, the use of high potency prostaglandin synthesis inhibitors has had opposite effects.

Commonly used non-steroidal anti-inflammatory analgesics have, as their basis of action, an inhibitory effect on prostaglandin synthesis. Depending on the type of analgesic used the inhibition can occur at different sites. There also exists a difference in the potency of the different prostaglandin inhibitors.

It is the purpose of this study to examine the following questions:

- 1) What effect does the peripheral prostaglandin inhibitor aspirin have on certain clinical and histologic aspects of orthodontic tooth movement?
- 2) Similarly, what effect does the central nervous system prostaglandin inhibitor acetaminophen have on the same parameters?
- 3) Is one drug preferable as an analgesic following orthodontic adjustment with respect to minimizing the effects of a prostaglandin inhibitor on tooth movement?

REVIEW OF THE LITERATURE

Prostaglandins are a family of lipids with significant pharmacological activity (1). They are composed of a basic 20 carbon atom skeleton, "prostanoic acid" (2). The prostaglandin synthetase enzyme system appears to be present throughout the animal kingdom manufacturing a variety of prostaglandins from essential fatty acid precursors (3). This enzyme system is not limited to any single organ system (4).

The multi-enzyme complex, prostaglandin synthetase, is found in the cell membrane (5). Prostaglandins are not stored within cells (6) therefore in situ production is required upon demand. Such demands for production arise from various types of stimulation including mechanical and chemical (7)(8)(9). The production site of prostaglandin is often the site of its rapid metabolism (10) with unmetabolized material being removed by the liver and lungs before it reaches arterial circulation (11). This suggests that prostaglandins are of little importance as circulating hormones. Their synthesis is generalized while their action and metabolism are highly localized.

One of the many roles that prostaglandins are believed to perform in cellular metabolism is that of inflammatory response mediator. Various types of prostaglandins have been shown to have a supportive role in the development of inflammation. They cause an increase in

vascular permeability (12), prolong erythema in human cutaneous blood vessels (13), induce wheel formation in human skin (14), and increase chemotaxis of polymorphonuclear leucocytes in rabbits (15). Prostaglandins may also have a role in wound healing (16).

The central and peripheral nervous systems are also biochemically linked to prostaglandins in that nerve stimulation results in prostaglandin formation (17)(18). The nerve activity-prostaglandin release phenomenon under diverse conditions suggests a fundamental role for prostaglandins in nerve and cell membrane physiology (19) particularly as it applies to self-regulation of synaptic pathways within the brain (20). It has been suggested that prostaglandins within the central nervous system act as modulators of synaptic activity (19).

Prostaglandins may act as extracellular first messengers (21) or as modulators of the effect that cyclic adenosine monophosphate (cAMP) has on target tissues (22). cAMP is presently considered to have a significant influence over the intracellular response to many hormones (23). They act as second messengers mediating the effects of the extracellular signal (first messenger) (24). The influence of prostaglandins on intracellular levels of cAMP is to either stimulate its synthesis or degradation depending on the tissue under consideration. The majority of tissue systems studied demonstrated an increase in the cAMP levels upon the addition of prostaglandins (16, 25-28).

The biology of tooth movement requires that three events take place:

- 1) Cellular disruption through a variety of stimulation pathways such as mechanical, chemical (inflammation) and/or electrical (piezo potentials) (29)(30)(31).
- 2) Formation and/or release of chemical messengers, modulators and mediators in response to the cellular disruption (21)(22)(32).
- 3) Translation of chemical signals into reactive mechanisms affecting bone resorption and deposition (33-37). Interference with any one of the above stated events may result in an absence of tooth movement.

In the biochemical realm of tooth movement prostaglandins appear to have an integral role. Demonstration of increased levels of prostaglandin during orthodontic tooth movement (38) are consistent with in vitro work on a variety of stressed cell membranes (29) in which the membranes react to compression with the formation and/or release of prostaglandins. Further evidence of this phenomenon is found in the prostaglandin mediated inflammatory response associated with experimental tooth movement (39) as well as in studies relating the rate of orthodontic tooth movement to the presence of synthetic prostaglandins or prostaglandin inhibitors.

In a series of experiments designed to study the clinical and histologic effects of locally injected prostaglandins, Yamasaki found that rat molars receiving no orthodontic force, displayed an increase in bone resorption and an increase in the number of

osteoclasts following local injections of prostaglandins (40). He found the rate of orthodontic tooth movement to double in monkeys following the administration of high, locally injected, concentrations of prostaglandin (41). He subsequently moved on to human clinical trials, with similar results utilizing the same methods. The rate of buccal movement of premolars was doubled while that of distal canine movement was increased 1.6-fold (42).

Further evidence for the role of prostaglandins in orthodontic tooth movement is seen in studies utilizing prostaglandin inhibitors. Chumbley and Tuncay, in an often cited article, found the prostaglandin inhibitor, indomethacin, to significantly slow the rate of orthodontic tooth movement in cats (43). Yamasaki observed reduced numbers of osteoclasts and reduced bone resorption in orthodontically treated rat molars following administration of indomethacin (40). Harris similarly found inhibition of prostaglandin synthesis, through administration of flurbiprofen, to reduce the number of osteoclasts observed while orthodontically moving rabbit teeth (44).

Clearly prostaglandins have a significant role in the functioning of biological systems. Their presence results in alteration of the immediate biochemical environment ultimately leading to a cellular response. That cellular response, in the case of tooth movement, is to facilitate bony remodeling. However, in the case of pain thresholds the effect of these locally active prostaglandins may have a less than desirable effect.

There exist three major groups of nerve fibers each distinct in its morphology, rate of transmission, and activation. The three groups are designated "A", "B", and "C". The "C" group, in humans, is almost exclusively activated by noxious stimuli. The noxious stimuli to be considered here is that which often times accompanies orthodontic appliance adjustment. Tooth movement has been shown to create an inflammatory response in the periodontal ligament (39). Prostaglandins, in addition to being mediators of the inflammatory response, are believed to exist in the inflammatory exudate (45). In humans prostaglandins have also been found to produce pain upon injection. In this situation the amount of pain perceived was dependent upon both the concentration of prostaglandin and the duration of its exposure to the tissue (46).

Recent evidence indicates that prostaglandins "sensitize" peripheral C nociceptive afferents lowering the pain thresholds (47) and effectively increasing pain sensitivity to chemical and mechanical stimulation (46). Therefore, interference with the synthesis of prostaglandin should result in an increase of the "C" fiber threshold, thereby reducing perceived pain.

Common analgesics of the salicylate family are inhibitors of the microsomal enzyme system "prostaglandin synthetase" (48-50). Evidence exists attesting to the potency differences among members in this family of drugs (51). Vane (49) found indomethacin to be 47 times as potent as aspirin and 1000 times as potent as salicylate.

Other investigations have found indomethacin to be 20 (52) to 250 (53) times as potent as aspirin in the total inhibition of prostaglandin synthesis. Indomethacin is the drug most commonly used in animal studies evaluating the effect that prostaglandin inhibitors have on orthodontic tooth movement while the much less potent inhibitor, aspirin, is consumed by humans for the purpose of analgesia.

In humans, three days of treatment with oral aspirin resulted in a 65-75% decrease in the prostaglandin content of seminal fluid (54). After six days of aspirin therapy the PGE levels remained low but began to rise. This suggests that prostaglandin synthesis "escapes" the inhibitory effect of aspirin. Further evidence that prostaglandin inhibition by aspirin is short lived is in the study of prostaglandin metabolites where excretion of PGE metabolic by-products decreases during aspirin therapy and returns to control levels two days after discontinuation of aspirin therapy (55).

All sites of action at which aspirin inhibits the initial enzyme in the prostaglandin biosynthesis pathway is considered to be "whole body". In other words, all sites of prostaglandin synthesis within the body are affected. This is in contrast to another commonly used non-steroidal anti-inflammatory drug, acetaminophen. This aniline derivative is almost as potent as aspirin in inhibiting prostaglandin synthesis in the CNS, but its peripheral inhibition of prostaglandin

synthesis is minimal, which may account for its lack of clinically significant anti-rheumatic or anti-inflammatory effects (56).

This difference in the site of action between aspirin and acetaminophen becomes important when one considers prostaglandins and their relationship with tooth movement and "pain". In the orthodontic event tooth movement is desired and this involves the presence of prostaglandins. The prostaglandins in turn sensitize the C nociceptive afferent nerves to the very mechanical and chemical environmental changes that orthodontic manipulation creates. Will analgesic drugs that inhibit prostaglandin synthesis at the site of this environmental change also inhibit the tooth movement induced by the mechanical and chemical environmental changes? If so, would the drug of choice be one that has its site of action away from the site of this environmental change?

Specifically, does a commonly used, peripheral "whole body" prostaglandin inhibitor, analgesic (aspirin) decrease the rate of orthodontic tooth movement as compared to a central nervous system prostaglandin inhibitor (acetaminophen)? If so, to what degree?

It is the hypothesis of this study that aspirin will reduce the rate, quantity and histologic evidence of tooth movement in experimental animals as compared to acetaminophen and controls.

MATERIALS AND METHODS

This study utilized 42 male Hartley guinea pigs obtained from Harlan Sprague Dawley, P.O. Box 29176, Indianapolis, IN 46229. Fourteen animals were utilized in each of two experimental groups and one control group. The animals were approximately six weeks old with an average weight of 360 grams. At this age the premaxillary suture is closed (57) and any lateral force applied to the maxillary incisors should result solely in movement of the incisors rather than separation of the premaxillary bones.

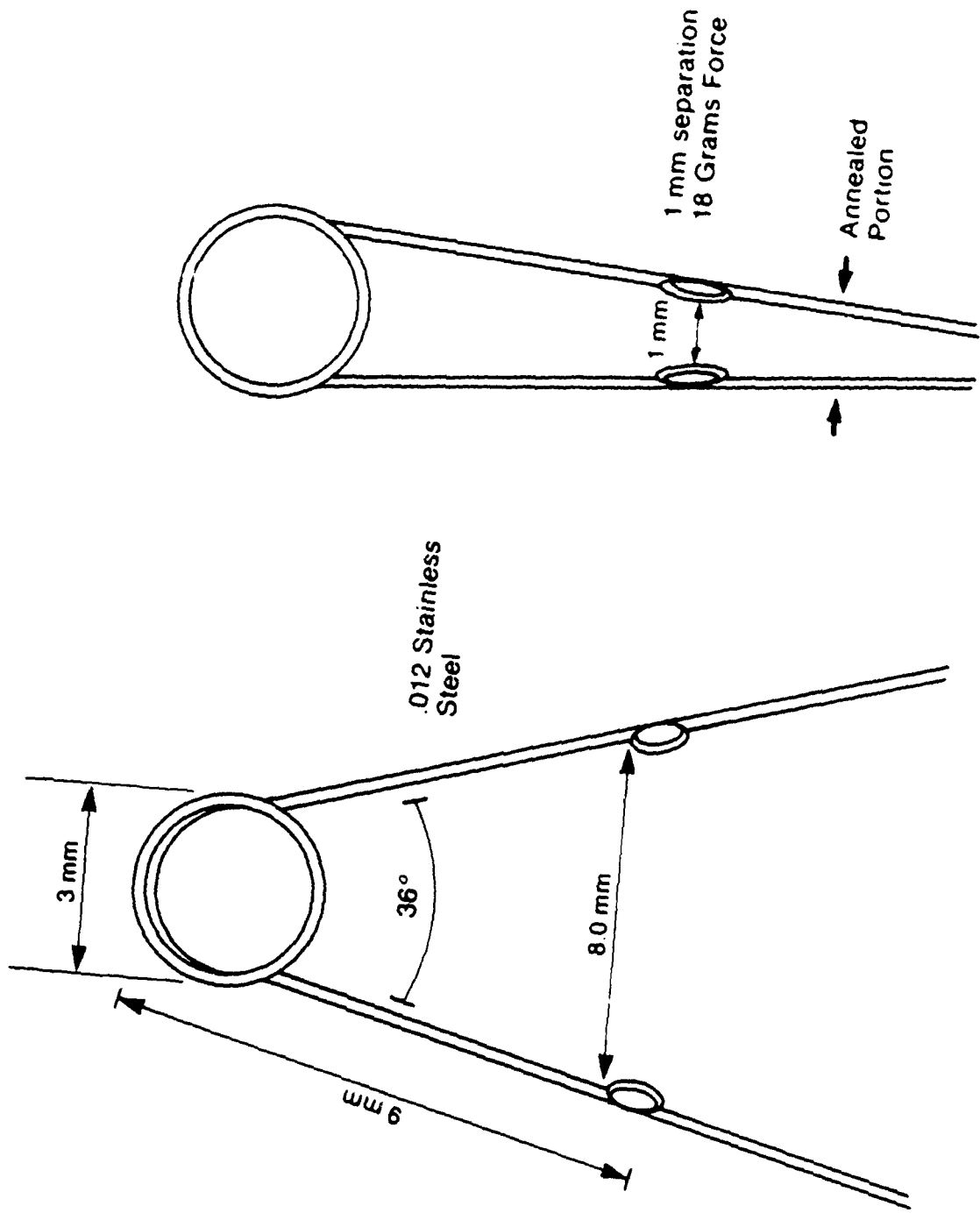
The animals were housed three or four to a 24" x 18" x 14" stainless steel cage. Vivarium temperature was maintained at a constant 78 degrees F. Water and commercial guinea pig feed were available ad libitum. The ease of handling and docile nature of guinea pigs, as noted by Stark (58), made them ideal subjects for this study.

Orthodontic Force System

The spring apparatus utilized for this study was similar to that described by Storey (57) in his descriptive series of studies on tooth movement in rats, rabbits and guinea pigs.

An improvement on Storey's spring design was made by Botting and Storey (59) in which the springs were placed intraorally to avoid

Figure 1. Drawing of the orthodontic coil spring utilized in this study. 18 grams of force were measured when the small coil stops were closed to a separation distance of 1 mm [adapted from Stark and Sinclair (58)].



The commonly used prostaglandin inhibiting analgesics utilized were:

- 1) Aspirin 325 mg tablets; Norwich Eaton Pharmaceuticals, Inc., Cincinnati, Ohio 45202.
- 2) Acetaminophen 325 mg tablets; Brand name Tylenol, McNeil Laboratories, Inc., Fort Washington, PA 19034.

In order to estimate the amount of aspirin required to produce an analgesic or therapeutic dose in guinea pigs an analogy between man and guinea pigs, with respect to warfarin elimination, was utilized. In man and guinea pigs warfarin and its metabolites are similarly metabolized and excreted in the urine (60). Man and guinea pigs experience similar biochemical elimination of the drug warfarin through displacement of warfarin bound to plasma protein. This facilitates uptake of warfarin by the liver where it is metabolized and eventually excreted in the urine (61). Therapeutic doses of non-steroidal anti-inflammatory drugs (NSAIDs) in man have no effect on the excretion of warfarin (62). To accelerate this elimination, man requires a larger than therapeutic dose of NSAIDs. It may then be reasonable to assume, given no other evidence, that a larger than therapeutic dose of salicylate would be required to accelerate warfarin elimination in guinea pigs. Wong et al. (61), found that 177.8 mg/kg of sodium salicylate resulted in an accelerated elimination of warfarin in the urine of guinea pigs. Based on this information the aspirin dose chosen for this study was 30% higher, (250 mg/kg), than the sodium salicylate dose used in Wong's study,

essentially guaranteeing an accelerated warfarin elimination response. Although this aspirin dosage will probably be larger than a therapeutic dose, it will nonetheless be lower in prostaglandin inhibition potency than previous tooth movement studies dealing with prostaglandin inhibition (40)(43).

To estimate the proper therapeutic dosage of acetaminophen in guinea pigs, the following conclusions from hepatotoxicity and metabolite studies were used.

- 1) Guinea pigs are quite resistant to acetaminophen induced hepatic necrosis.
- 2) Guinea pigs are sensitive to the analgesic properties of acetaminophen.
- 3) Acetaminophen dosages in excess of 200 mg/kg were found to produce marked hypothermia and some respiratory depression.
- 4) Orally administered acetaminophen demonstrates a rapid uptake and rapid formation of high plasma levels of metabolite (63)(64).

Based on these findings the dosage of acetaminophen chosen for this study was 100 mg/kg. It was concluded that this dosage would produce an adequate analgesic response while minimizing the adverse effects on the CNS.

Calcium carbonate was administered to the control group at a dosage of 100 mg/kg (Fisher Scientific Co., Fair Lawn, New Jersey).

Aspirin, acetaminophen and calcium carbonate, all white, water insoluble substances, were crushed to a similar consistency with a mortar and pestle. The powders were weighed and suspended in a 20%

sucrose solution utilizing a Corning PC-351 Hot Plate magnetic stirring apparatus. The sucrose was added to increase palatability of the suspension.

In order to determine the weight of powder necessary to maintain a constant volume of powder-sucrose solution mixture at the desired dosage, various amounts of powder and sucrose solution were placed in previously weighed plastic containers and allowed to evaporate, after which the residue was weighed.

- 1) 0.30 ml of 4.2 gram Calcium carbonate in 30 ml of 20% sucrose solution resulted in a 35 mg evaporated dose of powder.
- 2) 0.30 ml of 4.2 gram Acetaminophen in 30 ml of 20% sucrose solution resulted in a 34 mg evaporated dose of powder.
- 3) 0.30 ml of 9.4 gram Aspirin in 30 ml of 20% sucrose solution resulted in a 96 mg evaporated dose of powder.

Once the powders were completely suspended in the sucrose solution a 1 ml tuberculin syringe was used to draw up 0.30 ml of the suspension for administration to the animals.

Efforts taken to maintain a blind study were as follows.

- 1) utilization of similar looking drugs
- 2) constant volume of powder suspension administration
- 3) reference to the two experimental groups and one control group as Group I, Group II, and Group III with another person (CRK) the sole individual with the knowledge as to which group received which powder. After the drugs were weighed out they were given to (CRK) for group labeling and then returned to the

investigator for utilization. The group identity was not revealed to the investigator until completion of the study.

Experimental Procedure

Following a seven day acclimatization period the experimental procedures were begun. On the first day the guinea pigs were anesthetized by a combination of Ketamine hydrochloride, 35 mg/kg body weight, and Acepromazine maleate, 0.35 mg/kg body weight injected via an intraperitoneal route. Each animal was weighed and identified by a permanent dye marker to the fur on the back of the neck. A photograph of the guinea pig incisors prior to any manipulation can be seen in Figure 2. A small hole was drilled from labial to lingual in the center of both of the animal's two maxillary incisors at a distance of 2 mm from the incisal edge. The holes were cut with a 1/4 round bur in a high speed, air cooled, handpiece. Placement of the holes in this location avoided the pulp of the tooth by several millimeters while still leaving adequate tooth structure for normal incisal wear to the occlusal of the holes. The initial distance between the two holes was measured with the Helios dial calipers under 2X magnification and recorded.

For each animal a coil spring was selected at random and the wire distal to the coil stops was annealed. The coil spring was then placed intraorally and each leg was guided into its respective hole in the tooth from a lingual approach. The spring was drawn anteriorly until the coil stops rested against the lingual surface of

Figure 2. Photograph of the guinea pig incisors prior to any manipulation.



the incisors. The annealed portion of each spring leg, which projected forward from the labial surface of the incisors, was bent laterally and then distally back upon itself to help stabilize and secure the spring from dislodgement (Figures 3,4).

The animals were returned to their cages and observed until full recovery from the anesthesia was evident (60-90 minutes).

The initial drug dose was given to each animal after its recovery from anesthesia. Contents of a powder packet were suspended in 30 ml of the 20% sucrose solution with the magnetic stirrer. 0.30 ml of the suspension was then drawn up into a 1 ml tuberculin syringe. The first animal from the first cage was removed, the syringe tip placed at the back of the throat and the powder suspension injected. The animal was then placed back into the cage and observed to determine if the suspension was completely swallowed or expectorated.

Approximately 5% of the time any given animal would voluntarily or involuntarily expectorate the suspension at which time another full 0.30 ml of suspension would be administered. On the rare occasion that the second administration would be expectorated no further attempt would be made to give a third dose in order to minimize animal discomfort. No one group experienced more expectoration than the other two groups.

The same regimen was followed for all 14 animals in Group I. Analogous procedures were followed for Groups II and III. Doses were repeated at 12 hour intervals for 8 days.

Figure 3. Frontal view of guinea pig incisors immediately after placement of the orthodontic coil spring.

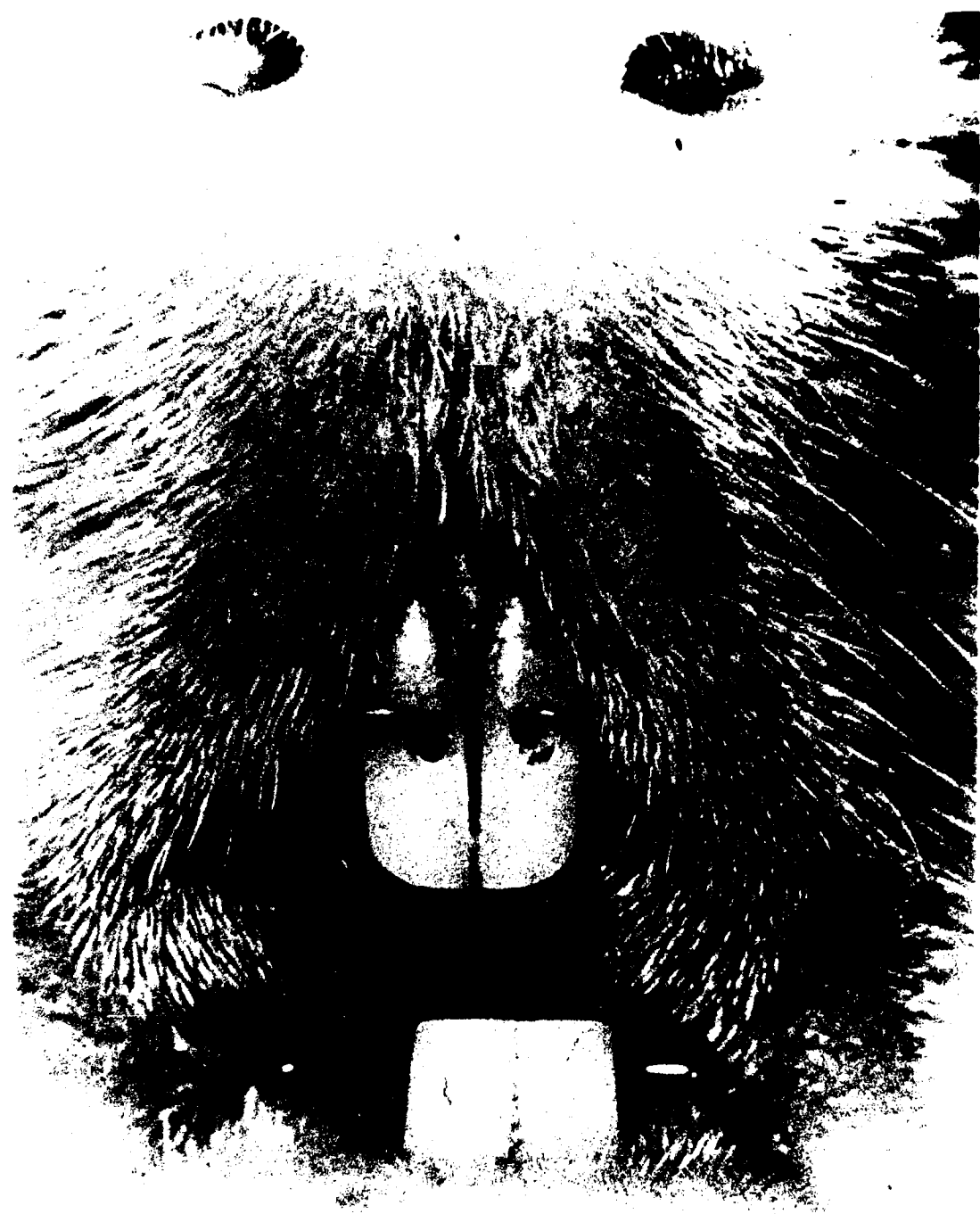
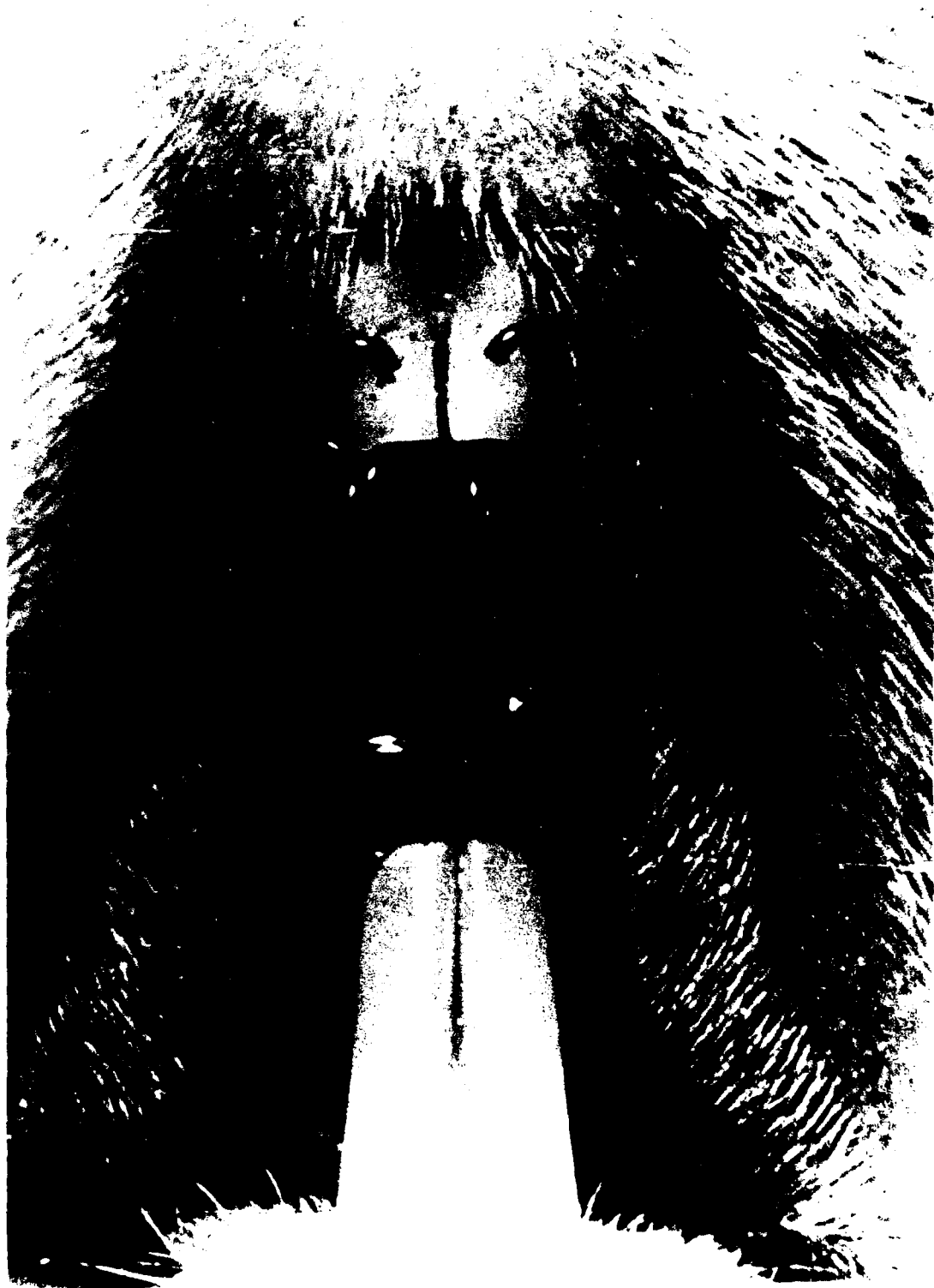


Figure 4. Intraoral view of the orthodontic coil spring.



The distance between the two holes in the guinea pig maxillary incisors was measured each evening during the 8 day testing period. The measurement landmarks were the most medial point on the medial margin of the holes in the left and right incisors. Accuracy of measurement was maximized by holding the animal and covering its eyes with the left hand. This calmed the animal and minimized its movement. The animal and Helios caliper were then held under a 2X magnifying glass and the distance between incisor holes measured (Figures 5,6). Each measurement was read to 0.05 mm and recorded, one time only, by a single investigator (CEB).

After 6 days the animals in Group III were experiencing a significant number of spring losses due to one leg of the spring passing through one of the incisors actually enlarging the hole laterally. This necessitated a change in protocol, with reduction in the number of days of observation from 10 to 8, in order to preserve enough animals in Group III to keep the study valid with respect to sample size.

The evening of the 8th day the animals were weighed, anesthetized with an intra peritoneal injection of 35 mg/kg of Ketamine hydrochloride and 0.35 mg/kg of Acepromazine maleate, and subsequently euthenized with an intracardiac injection of T-61 euthanasia solution (American Hoechst Corp., Somerville, NJ 08876).

A block section of the maxilla including experimental teeth and surrounding bone was then surgically removed within ten minutes of



Figure 6. Photograph of representative tooth movement at termination of the clinical portion of the study.



death and placed in Bouins fixative solution. The tissue preparation sequence can be found in Appendix A.

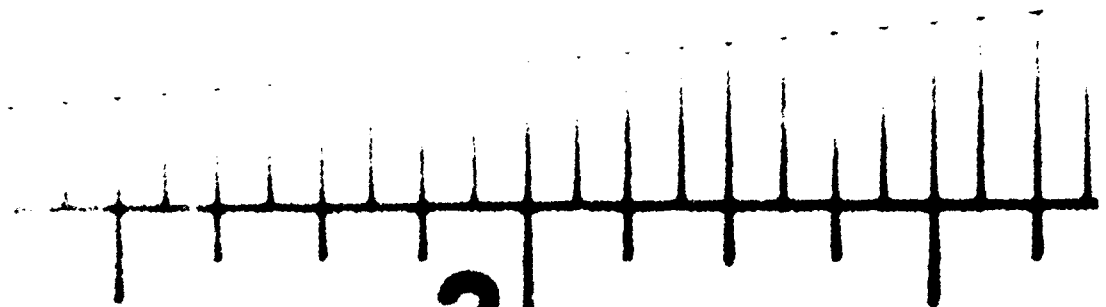
Following decalcification, and prior to the embedding stages, gross trimming of the specimens was accomplished. In order to maximize the amount of bone tissue around the teeth the most anterior portion of each specimen was trimmed to coincide with the area where the incisors became denuded of bone. The most posterior portion of each specimen was trimmed 2 mm posterior to the anterior trim incision. Embedding was then accomplished so that the most anterior portion of each specimen would be sectioned first (Figures 7,8). Cross sections were cut at 6 micron thicknesses on a Spencer 820 Microtome from anterior to posterior. Every 5th section was stained with hematoxylin and eosin. A total of 5 stained sections were utilized from each specimen, and osteoclast cell and nuclei counts were made from the 2nd, 4th, and 5th sections on each slide.

Histologic sections were examined and photographed with a Zeiss Transmitted-light Photomicroscope III.

Osteoclasts were counted on the resorptive side of the central incisors. Osteoclast counting was bounded anteriorly and posteriorly by lines tangent to the anterior and posterior surfaces of the tooth and perpendicular to the long, antero-posterior, axis of the tooth (Figure 9).

All counting observations were made with a 25X objective and 1.25X optovar resulting in a magnification factor of 100X. Osteoclasts were identified as cells with relatively large size,

Figure 7. Guinea pig maxilla marked prior to gross sectioning.



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Figure 8. Cross section of guinea pig maxilla. Each section is 2 mm thick anteroposteriorly.



8

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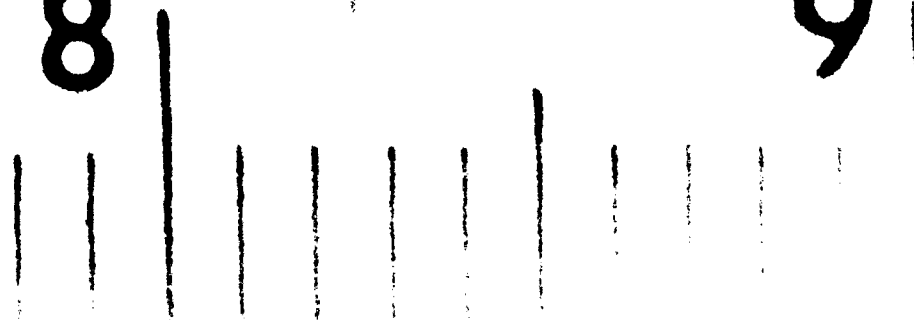


Figure 9. Illustration of the boundaries between which all histologic counts and measurements were made.



↑ Direction of
Tooth Movement

Posterior
Border →

Alv
Bone

PDL

←
Anterior
Border

foamy cytoplasm, association with areas of apparent bone resorption (lacunae), and predominantly multinucleated (Figures 10, 11). In addition to osteoclast counts the number of osteoclast nuclei was also recorded. Studies observing bone resorption, on the light microscope level, have shown osteoclastic activity to be better reflected in osteoclast size as determined by the number of nuclei present in each osteoclast rather than by the number of osteoclasts in a given area (65-67).

Linear bone surface dimensions were measured, within the above stated boundaries, under 10X magnification, utilizing the Bioquant software program, (R&M Biometrics, 5611 Ohio Avenue, Nashville, TN 37209), and a Compaq Desk Pro 286 12MHz PC. This allowed the establishment of a more precise ratio of osteoclasts/mm of linear bone surface and osteoclast nuclei/mm of linear bone surface.

Statistical examination of the clinical data consisted of:

- 1) test of parallelism - this examined the interaction between days and groups - if no significant interaction exists the velocity curve lines are considered parallel.
- 2) test for magnitude - this examines the magnitude of difference between parallel velocity curves.
- 3) cross-sectional examination of the data - this test looked for significant differences between groups at any given day of the study.



Figure 11. Representative osteoclast under high power (100X).



Statistical examination of the histologic data was done with an analysis of variance. The level of significance at which the null hypothesis would be rejected was set at $p < .05$.

RESULTS

Following completion of data collection, both clinical and histologic, the three groups were revealed to have been administered the following drugs:

- 1) Group I - Calcium Carbonate
- 2) Group II - Acetaminophen
- 3) Group III - Aspirin

Tooth Movement-Clinical Observations

Cumulative tooth movement for days 1-8 (Tables 1 and 2) were statistically analyzed. The test for parallelism displayed no significant interaction between days and groups, therefore, the linear regression lines for all three groups were considered parallel. When the test for magnitude of difference between the parallel linear regressions was performed it revealed no significant differences between the three groups. A cross-sectional examination for each individual day of the study also failed to disclose any significant differences between the three groups. Although the daily incremental changes in tooth movement displayed significant statistical differences among the three groups, these differences showed no pattern of consistency.

As can be seen from the velocity curve (Figure 12), the tooth movement pattern in this study was similar to that noted in other

Table 1. Results - Cumulative Tooth Movement in mm

ID	Group	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Change
011AR1	1	1.10	3.05	3.45	3.60	3.75	3.55	4.05	4.35	4.80	3.70
021AB1	1	1.35	3.65	3.80	4.10	4.10	4.20	4.20	4.20	4.55	3.20
031AR2	1	1.85	3.60	3.70	4.00	4.30	4.30	4.30	4.60	5.10	3.25
041AB2	1	1.15	3.30	3.70	4.05	4.30	4.60	4.65	4.80	5.65	4.50
051AG2	1	1.40	3.35	3.80	4.00	4.05	4.05	4.15	4.20	5.05	3.65
061AW2	1	1.50	3.70	4.10	4.10	4.25	4.35	4.40	4.75	5.95	4.45
071BR1	1	1.25	3.30	3.50	3.65	3.85	4.00	4.10	4.55	5.05	3.80
081BB1	1	1.45	3.50	3.60	3.85	3.95	4.20	4.55	4.90	5.25	3.80
091BG1	1	1.50	3.45	3.55	3.90	4.20	4.50	4.65	5.00	5.55	4.05
101BB2	1	1.55	2.90	3.20	3.50	3.75	4.05	4.30	4.60	4.80	2.25
111BG2	1	1.60	3.40	3.75	3.95	4.15	4.20	4.45	4.50	4.50	2.90
121BW2	1	1.25	3.50	4.00	4.55	4.75	4.75	4.95	5.75	6.10	4.85
132AR1	2	1.15	3.25	3.60	3.65	3.75	3.75	3.95	3.95	4.25	3.10
142AB1	2	1.35	2.85	3.05	3.25	3.55	3.95	4.10	4.25	4.25	2.90
152AG1	2	1.55	3.80	4.40	4.55	4.60	5.00	5.10	5.20	5.40	3.85

Table 1. (Continued)

ID	Group	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Change
162AR2	2	1.30	3.35	3.60	3.75	3.85	3.85	3.85	4.20	5.20	3.90
172AB2	2	1.30	3.00	3.35	3.60	3.95	4.15	4.15	4.40	4.40	3.10
182AG2	2	1.15	2.85	3.15	3.50	3.65	3.85	3.95	3.95	4.20	3.05
192AW2	2	1.50	3.00	3.30	3.45	3.60	3.80	4.05	4.40	4.55	3.05
202BR1	2	1.25	3.15	3.35	3.80	4.20	4.20	4.35	5.00	5.55	4.30
212BB1	2	1.30	3.10	3.40	3.80	3.95	4.10	4.10	4.65	5.15	3.85
222BG1	2	1.60	4.00	4.00	4.20	4.60	4.65	4.65	4.75	5.15	3.55
232BR2	2	1.25	3.40	3.75	3.95	4.50	4.90	5.40	5.75	6.10	4.85
242BB2	2	1.50	2.60	3.30	3.60	3.90	4.20	4.20	4.40	5.00	3.50
252BW2	2	1.35	3.10	3.65	4.00	4.45	4.60	4.75	4.90	5.35	4.00
263AG1	3	1.50	2.90	3.30	3.55	3.85	3.85	3.85	4.25	4.45	2.95
273AG2	3	1.55	3.60	3.65	3.80	4.10	4.25	4.25	5.10	5.80	4.25
283BB1	3	1.45	3.20	3.60	3.75	3.95	3.95	4.70	4.90	4.90	3.45
293BG1	3	1.35	2.80	3.55	3.75	3.80	3.80	4.35	4.55	4.75	3.40

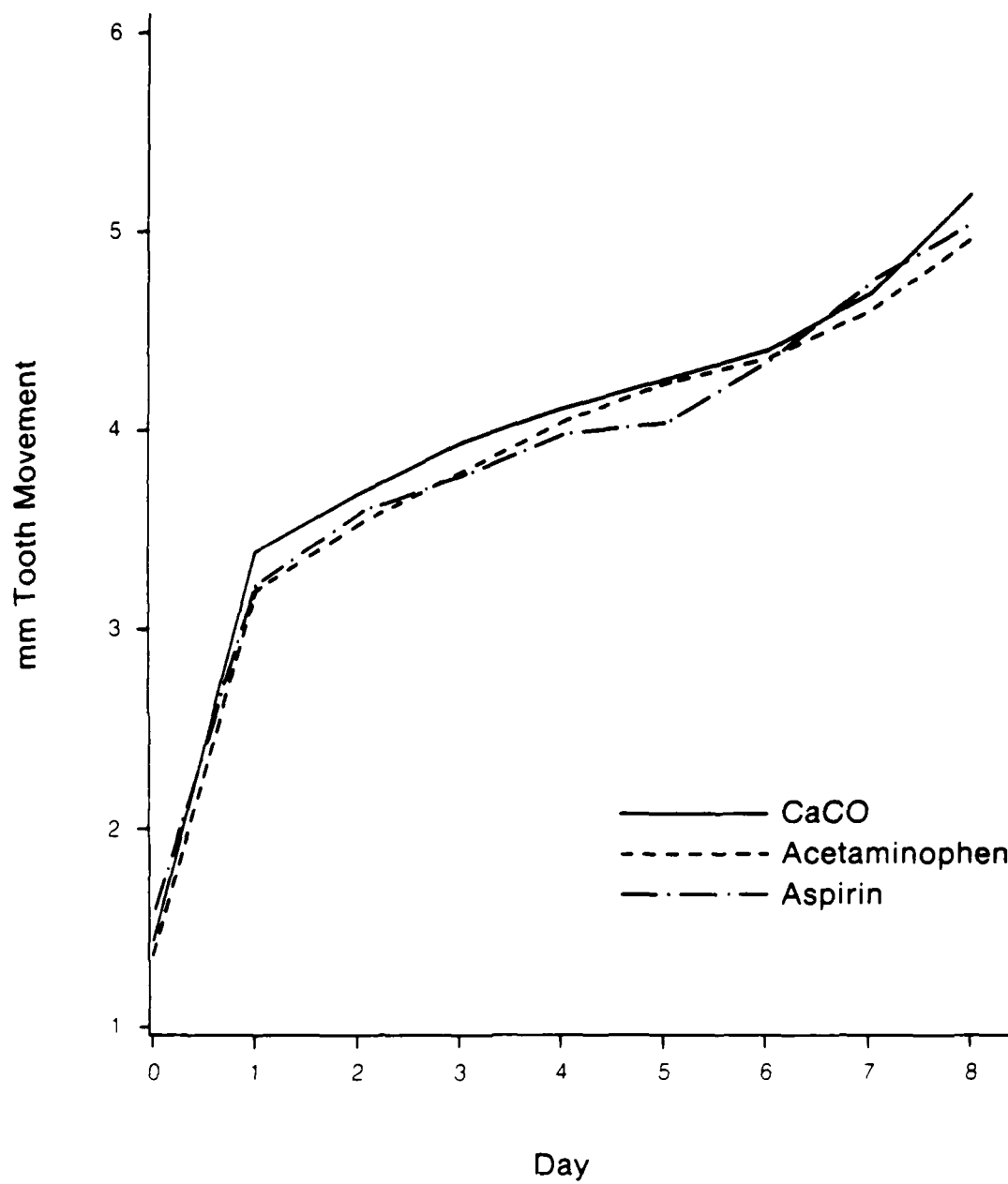
Table 1. (Continued)

ID	Group	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Change
303BR2	3	1.70	3.55	3.80	3.95	4.20	4.25	4.30	4.65	5.00	3.30
313BG2	3	1.70	3.25	3.60	3.80	4.00	4.10	4.60	5.05	5.35	3.65

Table 2. Cumulative Amounts of Tooth Movement in mm

Day	Control Group ($\bar{x} \pm SD$) n=12	Acetaminophen Group ($\bar{x} \pm SD$) n=13	Aspirin Group ($\bar{x} \pm SD$) n=6
Start	1.41 \pm 0.21	1.35 \pm 0.15	1.54 \pm 0.14
1	3.39 \pm 0.24	3.19 \pm 0.38	3.22 \pm 0.33
2	3.68 \pm 0.24	3.53 \pm 0.37	3.58 \pm 0.16
3	3.94 \pm 0.28	3.78 \pm 0.34	3.77 \pm 0.13
4	4.12 \pm 0.28	4.04 \pm 0.38	3.98 \pm 0.15
5	4.26 \pm 0.25	4.23 \pm 0.42	4.03 \pm 0.20
6	4.40 \pm 0.27	4.35 \pm 0.48	4.34 \pm 0.30
7	4.68 \pm 0.42	4.60 \pm 0.52	4.75 \pm 0.33
8	5.20 \pm 0.52	4.97 \pm 0.59	5.04 \pm 0.47
total change	3.78 \pm 0.59	3.62 \pm 0.58	3.50 \pm 0.43

Figure 12. Velocity curve of tooth movement.



mammals, with a period of initial rapid tooth movement, caused by compression of the periodontal ligament, followed by a more gradual increase in movement.

Loss of experimental animals due to problems with the orthodontic torsion spring occurred in all three groups. Group I (CaCO₃) experienced the loss of two experimental animals due to fracture of one of the incisors in the area of the spring hole. Group II (Acetaminophen) lost one experimental animal for the same reason. However, Group III (Aspirin) suffered the loss of eight members due to one leg of the spring actually passing through one of the incisors rendering the spring useless. It was for this reason that the length of the study was shortened to 8 days versus 10 days as originally planned.

Tooth Movement-Histologic Observations

Low power examination of histologic sections displayed the expected bone deposition in areas of tension and bone resorption in areas of pressure, particularly in the antero-lateral areas under observation for this study. No areas of cementum resorption were noted in the sections examined. Based on the number of inflammatory cells present inflammation of the periodontal ligament and surrounding bone was minimal.

Data, and its analysis, from histologic examinations can be seen in Tables 3-5. Analysis of variance was used to test these data for significant differences among the three groups. No differences were

Table 3. Results of Histologic Examination

ID	Group	Cell Count		Nuclei Count		Length in mm	
		R	L	R	L	R	L
1A1B	1	8	2	28	3	3.36	3.42
1A1R	1	4	20	11	85	2.90	2.45
1A2G	1	2	3	5	13	1.60	3.08
1A2R	1	3	7	6	26	2.44	2.82
1A2W	1	3	8	15	26	1.31	3.36
1B1B	1	5	7	17	14	1.85	3.07
1B1G	1	6	7	14	27	4.15	3.79
1B1R	1	11	9	34	19	2.14	3.31
1B2G	1	8	0	20	0	4.53	0.00
1B2B	1	2	9	5	26	4.19	3.88
1B2W	1	8	11	22	31	3.76	1.27
2A1R	2	2	1	8	3	4.25	3.28
2A1G	2	2	8	4	35	3.75	5.35
2A2B	2	8	3	20	7	2.07	3.52
2A2W	2	10	13	32	35	3.66	3.21
2A2R	2	4	8	11	25	3.85	3.96
2A2G	2	5	3	17	7	3.24	2.68
2B1B	2	6	11	14	31	2.62	3.46
2B1G	2	7	6	21	20	3.47	3.87
2B1R	2	1	1	2	3	3.28	2.65

Table 3. (Continued)

ID	Group	Cell Count		Nuclei Count		Length in mm	
		R	L	R	L	R	L
2B2W	2	4	5	6	13	2.83	5.12
2B2B	2	0	6	0	23	3.85	3.50
2B2R	2	7	7	15	26	4.05	2.76
3B1B	3	4	3	13	6	3.04	3.84
3A1G	3	4	7	11	17	3.69	3.07
3A2G	3	13	0	44	0	2.72	0.00
3B1G	3	2	1	5	2	3.17	4.06
3B2G	3	10	0	24	0	3.33	0.00
3B2R	3	4	6	15	11	2.01	2.74

Group 1 - CaCO_3

Group 2 - Acetaminophen

Group 3 - Aspirin

Table 4. Daily Incremental Amounts of Tooth Movement

Day	Control Group ($\bar{x} \pm SD$)	Acetaminophen Group ($\bar{x} \pm SD$)	Aspirin Group ($\bar{x} \pm SD$)
1	1.98 \pm 0.25	1.84 \pm 0.34	1.68 \pm 0.23
2	0.29 \pm 0.14	0.34 \pm 0.18	0.37 \pm 0.21
3	0.26 \pm 0.13	0.25 \pm 0.11	0.18 \pm 0.04
4	0.18 \pm 0.09	0.27 \pm 0.15	0.22 \pm 0.09
5	0.15 \pm 0.12	0.19 \pm 0.15	0.05 \pm 0.06
6	0.13 \pm 0.11	0.12 \pm 0.14	0.31 \pm 0.31
7	0.29 \pm 0.21	0.25 \pm 0.19	0.41 \pm 0.22
8	0.51 \pm 0.31	0.37 \pm 0.26	0.29 \pm 0.21

Table 5. Ratios of (A) Number of Osteoclasts/Linear Bone Surface
(B) Number of Osteoclast Nuclei/Linear Bone Surface

	Control ($\bar{x} \pm SD$)	Acetaminophen ($\bar{x} \pm SD$)	Aspirin ($\bar{x} \pm SD$)
(A)	2.05 \pm 0.55	1.51 \pm 0.73	2.50 \pm 1.20
(B)	6.56 \pm 2.54	4.16 \pm 1.88	5.57 \pm 3.21

found. Bar graphs were constructed from the histologic data by taking the ratio of right and left osteoclast cell count means to right and left linear bone measurement means (Figure 13) and right and left osteoclast nuclei count means to right and left linear bone measurement means (Figure 14). Although the lack of statistical differences between the three groups is not obvious from these graphs the lack of an apparent trend is evident.

Figure 13. Bar graphs depicting the ratio of osteoclast cell counts to length measurements for each of the three groups.

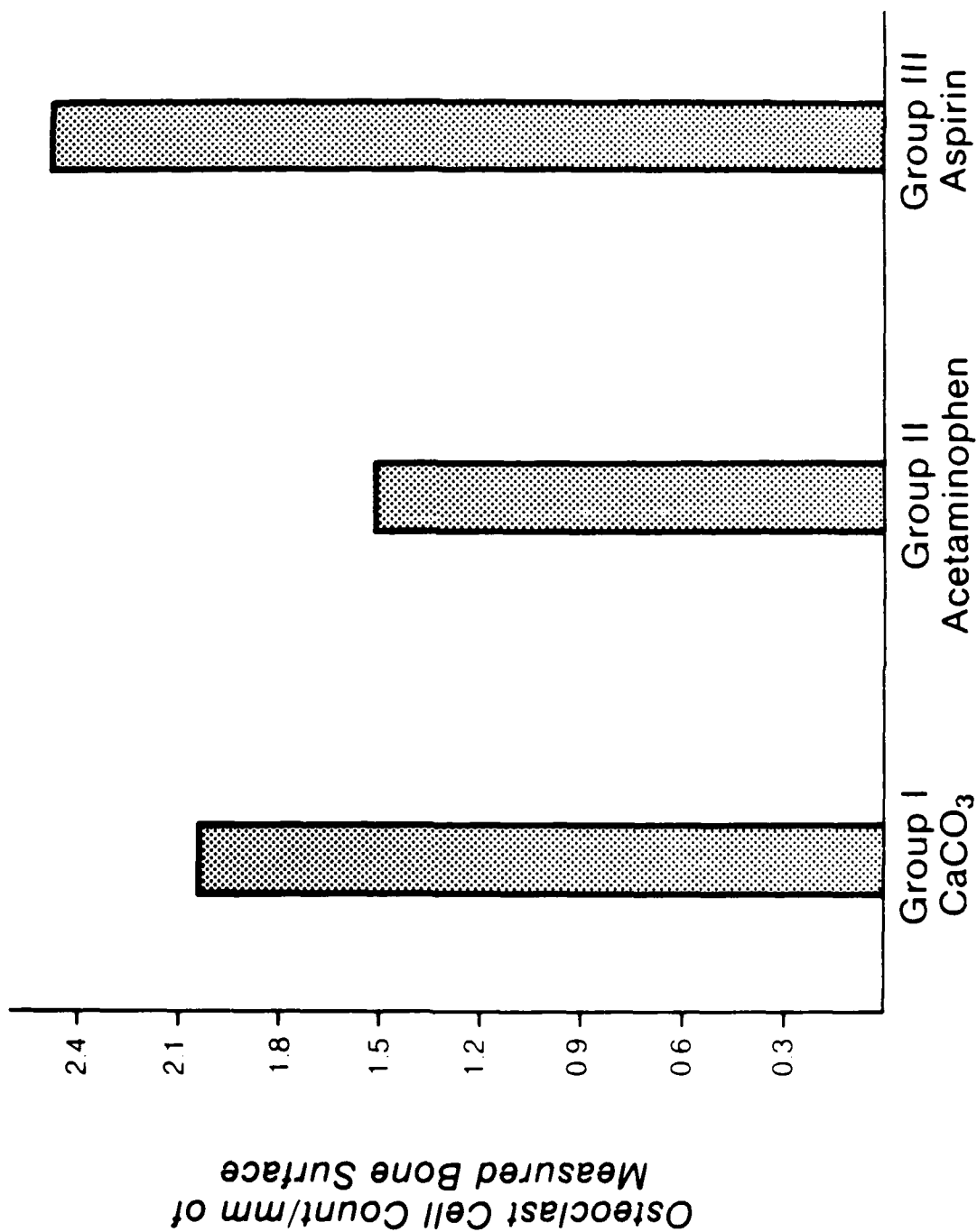
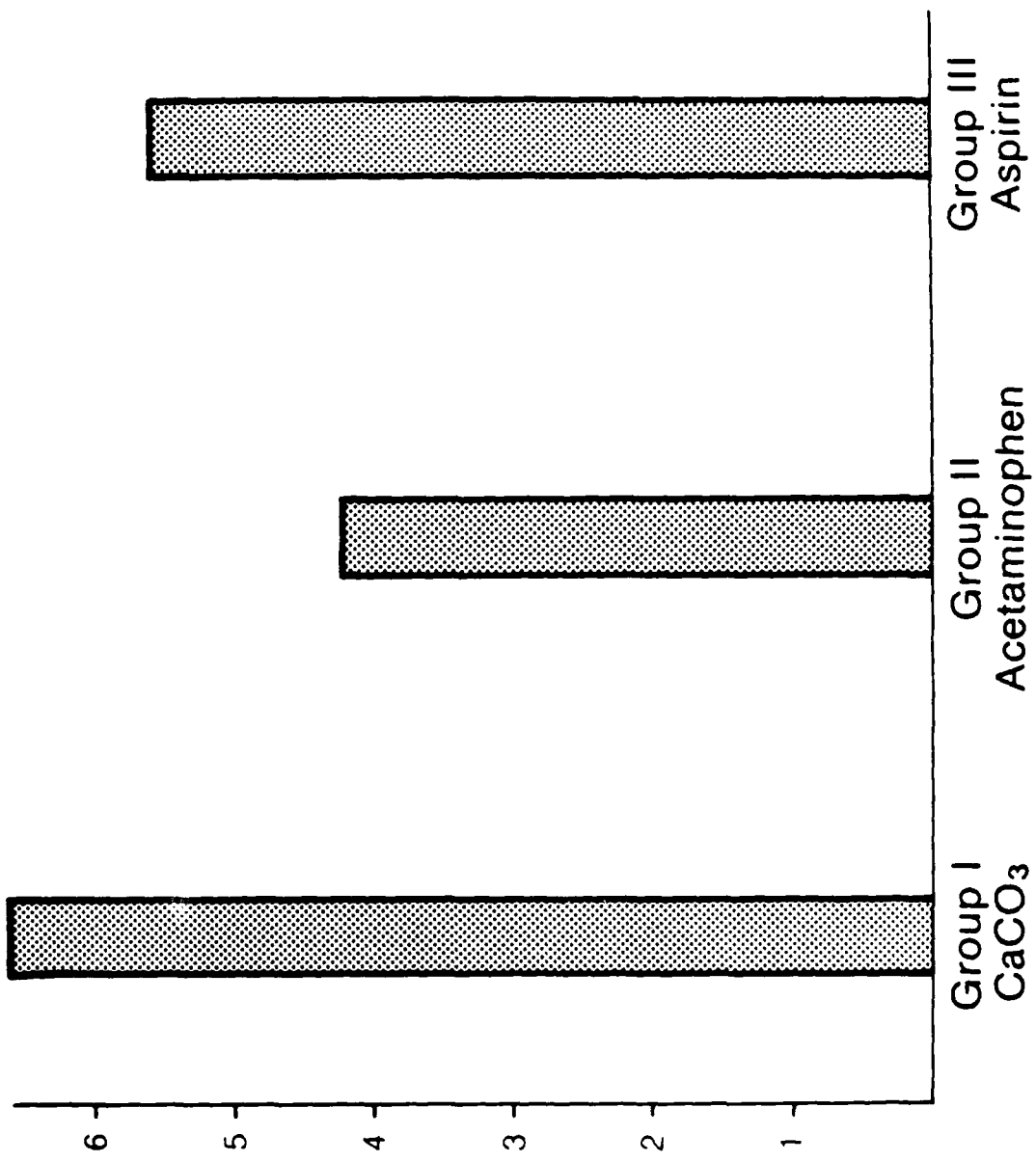


Figure 14. Bar graphs depicting the ratio of osteoclast nuclei counts to length measurements for each of the three groups.



Osteoclast Nuclei Count/mm of Measured Bone Surface

DISCUSSION

It was hypothesized that animals dosed with aspirin would exhibit both clinical and histologic evidence of decreased tooth movement while controls and those receiving acetaminophen would lack such a response. This was not the case in guinea pigs as no statistically significant differences, be they gross tooth movement or osteoclast and nuclei counts, were revealed among the three groups. Past studies have clearly demonstrated the effect that potent prostaglandin inhibitors have on the parameters examined in this study (40)(43). However, Sandy and Harris (44), in their rabbit study utilizing the prostaglandin inhibitor flurbiprophen found no statistically significant difference between control and experimental animals when examining gross tooth movement although statistically significant differences did exist in osteoclast cell counts. This raises the question as to what the lower threshold of osteoclast numbers, or their activity, might be for a significant alteration in observed clinical tooth movement. Past studies, utilizing potent prostaglandin inhibitors, found tooth movement to continue to occur, at decreased amounts and rates, despite complete blockage of prostaglandin synthesis (40)(43)(44). This argues for other biochemical pathways through which tooth movement is accomplished. Nevertheless, the abundance of evidence suggests that prostaglandins do play a

significant role in tooth movement. The present study does not contradict this evidence neither does it offer support. Specific efforts were made to give prostaglandin inhibiting drugs in doses considered to be in the therapeutic range and thereby obtain information concerning the effect that these inhibitors, at these doses, had on tooth movement. It was not the intention of this study to produce 100% prostaglandin synthesis inhibition, nor was it intended to inhibit synthesis for an inordinate amount of time.

In a study by White (68), a reduction in orthodontic pain was experienced by 63% of patients chewing a combination of gum and aspirin. What differential effect the chewing action or aspirin had on the reduction of discomfort is not known. What is known to most individuals dispensing orthodontic treatment is that taking over-the-counter pain medications is not an unusual occurrence in patients undergoing orthodontic therapy. This consumption consists of therapeutic doses over a highly variable, but usually short, period of time depending on the individual under consideration.

Acetaminophen, which was not predicted to have an effect on orthodontic tooth movement in this study, due to the location of prostaglandin synthesis inhibition, had no effect that was statistically different from the controls. Aspirin also failed to show any statistically significant differences in tooth movement from controls, although one would suspect there should be based on the location of it's biochemical action.

This study progressed with high investigator confidence in the accuracy of measurement through the use of easily controlled experimental animals and measurement magnification. Expectoration of the drugs upon administration was rare, therefore it was assumed that an adequate dosage was maintained throughout the study. Obviously, maintaining a blind study was not possible due to the large quantity of aspirin that was required to achieve a therapeutic dose when compared to CaCO_3 and acetaminophen. For this reason the aspirin group was properly guessed at from the beginning. However, the acetaminophen and control groups were indistinguishable. Reliability of this study is, therefore, suspect in light of the inability to maintain a completely blind experimental situation. Regardless, the investigator tried to remain objective.

The loss of a large number of springs in the aspirin group can be explained by the acidic environment caused by oral administration of the acetyl salicylic acid. Possible ways to avoid this problem are:

- 1) administer the aspirin via gastric tube bypassing contact of the drug with the teeth.
- 2) administer the aspirin in a fashion similar to that in this study followed by a copious rinsing of the incisors, particularly around the spring holes.
- 3) place corrosion resistant sleeves in the spring holes prior to placement of the springs.

The original protocol for this study called for a two stage experiment with 7 animals in each of 6 unique situations. As this protocol could not be followed, due to the spring difficulties, 12 animals ended up in the control group, 13 animals in the acetaminophen group and 6 animals in the aspirin group. This loss of animals from the experimental pool was regrettable but the remaining sample sizes were believed to produce valid data.

From the results of this study, it appears to be highly improbable that therapeutic doses of aspirin or acetaminophen would have a significant effect on tooth movement. When considering analgesics for post orthodontic adjustment discomfort the dosage is low and the duration of its consumption is short.

Future work in this area could involve a refinement and repetition of this study with steps taken to guard against loss of springs, in ways previously mentioned, as well as use of a third party to administer the drugs, in order to maintain a blind study. In this day of prophylactic aspirin consumption, by some individuals, it might be advantageous to know the effects of this consumption on prostaglandin levels and in turn the effects on tooth movement.

If future studies in this area are in agreement with the results presented here, then efforts should focus on the promotion of tooth movement through the use of exogenous prostaglandins. If not in agreement, a more in depth analysis of the effects of aspirin on orthodontic tooth movement is warranted.

Given the influence that prostaglandins have on tooth movement, it seems reasonable to recommend against the use of prostaglandin inhibitors for the relief of orthodontic discomfort. However, the results of this study can justify no such recommendation.

CONCLUSIONS

- 1) Administration of therapeutic doses of aspirin and acetaminophen over an 8-day period resulted in no significant differences in the rate or amount of orthodontic tooth movement when compared to control animals.
- 2) Histologic observation revealed no statistically significant differences between the aspirin, acetaminophen and control groups with respect to osteoclast counts/mm of pressure side linear bone surface and osteoclast nuclei counts/mm of pressure side linear bone surface.
- 3) Therapeutic doses of prostaglandin synthesis inhibiting drugs may be sufficient to increase pain thresholds but may be insufficient to cause a clinical or histologic alteration in orthodontic tooth movement.
- 4) The information obtained from this study does not contraindicate the use of prostaglandin inhibitors by orthodontic patients on the possibility that tooth movement will be significantly slowed down by consumption of the drugs.

APPENDIX A

HISTOLOGICAL PREPARATION SEQUENCE

- 1) Bouins fixative-----24 hrs
- 2) Decalcification with SSFA-----7 days
- 3) Radiographs every other day
- 4) Gross trimming
- 5) Wash in 70% Alcohol-----12 hrs
- 6) Wash in 70% Alcohol-----12 hrs
- 7) Wash in 90% Alcohol-----12 hrs
- 8) Absolute Alcohol I----- 8 hrs
- 9) Absolute Alcohol II----- 8 hrs
- 10) Absolute Alcohol III----- 8 hrs
- 11) Methyl Benzoate--3 changes over 18-24 hrs
- 12) Methyl Cellodin-----24 hrs +
- 13) Blot dry benzene-----1 hr (2) changes
- 14) B. wax-----1/2-1 hr
- 15) Maintain at 58 degrees until molten
- 16) Wax I-----1 hr
- 17) Wax II-----1 1/2 hrs vacuum
- 18) Wax III-----1 1/2 hrs vacuum

APPENDIX B

ANIMAL WEIGHTS

Initial Weight--Final Weight in grams

ID			
IA1R	---	349	----- 366
IA1B	---	379	----- 457
IA2R	---	363	----- 423
IA2B	---	350	----- 418
IA2G	---	367	----- 419
IA2W	---	348	----- 382
IB1R	---	356	----- 412
IB1B	---	350	----- 408
IB1G	---	386	----- 472
IB2B	---	356	----- 409
IB2G	---	365	----- 412
IB2W	---	392	----- 452
IIA1R	--	366	----- 426
IIA1B	--	373	----- 427
IIA1G	--	349	----- 384
IIA2R	--	368	----- 430
IIA2B	--	385	----- 450
IIA2G	--	326	----- 378
IIA2W	--	369	----- 402
IIB1R	--	372	----- 426
IIB1B	--	340	----- 400
IIB1G	--	352	----- 424
IIB2R	--	318	----- 357
IIB2B	--	370	----- 424
IIB2W	--	257	----- 433
IIIA1G	-	352	----- 392
IIIA2G	-	374	----- 432
IIIB1B	-	374	----- 433
IIIB1G	-	354	----- 395
IIIB2R	-	360	----- 431
IIIB2G	-	348	----- 376

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