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THE EFFECT OF PIRIPROST AND INDOMETHACIN ON PRESSURE INDUCED CALVARIAL REMODELING ALONG MIDSAGITTAL SUTURE OF THE RAT

by

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Abstract

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Leukotrienes (LTs) are products of 5-lipoxygenase pathway, a separate but related pathway to cyclooxygenase producing prostanoids in arachidonic acid cascade. LTs have been identified as important mediators of inflammation. Previous research (Collins 1986) has demonstrated that LTB_4 and LTC_4 also served as important mediators of sutural remodeling. Histological results have shown that the groups treated with/without sutural stress and piriprost (U-60,257, a 5-lipoxygenase inhibitor) had cuboidal and dark stained cells found in the midsagittal sutures of rats. These findings provoked some questions about the ability of piriprost to stop resorptive system and to initiate depository system by depressing LTs contents.

The present study was performed by setting up 6 groups of female Sprague-Dawley rats with 6 animals in each group. A two-phased experimentwas conducted in which the resorptive system in the midsagittal area was induced in the first 3-day phase, and then piriprost or piriprost/indomethacin mixture was injected to evaluate the sutural responses in terms of LTs and PGE_2 contents and histological behavior.

The designs of the 6 groups were as follow:

A - 6 days osteotomy on the midsagittal suture

B - 6 days same osteotomy treatment plus piriprost injection (10 mg/kg body weight) for the last 3 days

C - 3 days same osteotomy plus 20 g compressive force

D - Same condition with C but 6 days experiment

E - Same condition with D plus piriprost injection for the last 3 days

F - Same condition with E plus indomethacin (1 mg/kg body weight) injection together with piriprost

At the end of 6-day experimental period, levels of LTB_4, LTC_4 and prostaglandin E_2 (PGE_2) expressed as weight/mg of noncollagenous protein, were measured by radioimmunoassay (RIA) specific for each metabolite.

cells might be due to the blockage of necessary stimulating message from the cellular surface.

DEDICATED

TO

Sheyuan and Mary Shiao,

My parents, who have provided me tremendous

love and unselfish sacrifice through the years.

AND TO

My Sister and Brothers,

for their encouragements and pushes

that were essential to make my dream come true.

Finally, to my 5 classmates, Craig, Derya, Joe, Michael and Ioannis, because of your wonderful friendship and good cooperation I feel so joyful and satisfied in the first 2 years of life in the U.S.A.

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Introduction

Effect of local mediators such as PGE_2 and cAMP on bone remodeling has been studied for years by either injecting the mediators to the experimental areas or applying mechanical force on bony models to provoke and evaluate the mediators. According to many studies (Marcus et al. 1977, Heersche, 1978, Davidovitch et al. 1980, and Collins 1986), cAMP and PGE_2 are more likely common mediators to a variety of stimuli. For example, either compression or tension force can elevate PGE concentration in bony suture area. If so, what are the specific mediator(s) to a specific stimulation? Are there any other mediators working with bone remodeling? These questions may remain undiscovered for a while. In 1986 a new group of possible mediators, leukotrienes which are another group of arachidonic acid metabolites, have been studied (Figure 1, Figure 2) in sutural remodeling in their effects and concentration by Collins (1986). He found leukotriene B_4 level (LTB_4) is significantly reduced both in pressure and tension applied bony suture. While LTC_4 level is markedly increased in pressure groups, but little change in tension groups. In addition to the finding of relationship between stress application and leukotrienes concentration, he also observed an interesting histology

My experimental design is basically to evaluate the possible reverse effect of piriprost on the resorption induced sutural area under compressive force. And I am also interested in studying the combined effect of indomethacin and piriprost to the remodeling suture. These studies are evaluated by both histological observation and biochemical analysis of contents of leukotrienes and prostaglandin E_2 in the interested sutural area.

(1978), Davidovitch et al. (1980) have shown that cAMP may be a common mediator to a variety of stimuli such as parathyroid hormone, calcitonin and electrical current. As we know, the above hormones or stimuli can have different actions on bone remodeling while still have the same effect of raising cAMP content. Radulovich (1985) used indomethacin to study the role of PGE_2 and cAMP in the stress applied midsagittal suture of the rats. He found that both cAMP and PGE_2 were significantly elevated over control, and suppression of PGE_2 response by indomethacin reduced cAMP level by half in tension group but had no change in cAMP level in pressure group. This suggested that cAMP is a likely mediator of bone cell function during the remodeling process and has not related much to PGE_2 function. In other words, PGE_2 does not influence cAMP level directly.

Goodson et al. (1972) injected PGE_1 under the skin overlying the calvarium of adult rats and produced a visible resorptive lesion within the bone in seven days. They also observed that the resorptive process was characterized by fibrous replacement of bone matrix and by increased vascularity. This was probably the first descripton of resorptive effect of bone of PGE_1 in vivo.

Yamasaki et al (1982) did an experiment in monkeys in which they administered PGE_1, or PGE_2 locally combined with orthodontic tooth movement and found that the tooth movement was double the rate compared to the control.

Two years later (1984) he used volunteer patients and injected PGE_1 in the gingiva and again found that the tooth movement was almost double the rate compared to the control. With regards to inhibitors of PGs., Yamasaki et al (1980) demonstrated a dose related inhibition of osteoclast formation when indomethacin was administered prior to and during tooth movement. Sandy and Harris (1984) used flurbiprofen, a non-steroid anti-inflammatory drug and prostaglandin cyclooxygenase inhibitor, in New Zealand White rabbits and found inhibition of osteoclastic activity and bone resorption. Parker, (unpublished 1985) concluded from his study that indomethacin treated midsagittal sutures of rats reduced osteoclastic activities in the compressive force applied suture area. All observations strongly suggested that osteoclasts formation is positively related to the presence of prostaglandins (PGs).

Although PGs seem to favor bone resorption, many recent experiments and observations support the evidence the PGs stimulate bone formation in vivo. Baron et al (1978) injected PGE_1 locally to the adult rat calvaria daily and cuased periosteal bone formation. Ueno et al (1985) injected graded doses of PGE_2 to rapidly growing weanling rats and stimulated the replication and differentiation of osteoblast precursors and enhance the formation of metaphyseal woven bome. PGs have been

Figure 1 Origin of Arachidonic Acid and Its Metabolic Products. (Bengt Samuelsson 1983)

Arachidonic acid is normally stored in biological membrane structures and can be released through activation of a hydrolytic system by variety of stimuli. Depending on the availability of active enzymes in the stimulated cell arachidonic acid is converted into one or several biologically active molecules. A variety of stimuli can thus be converted into a multitude of compounds which can be used to regulate or mediate various cell functions.

Aspirin, indomethacin and piriprost serve as inhibitors of the two main arachidonic acid metabolic pathways.

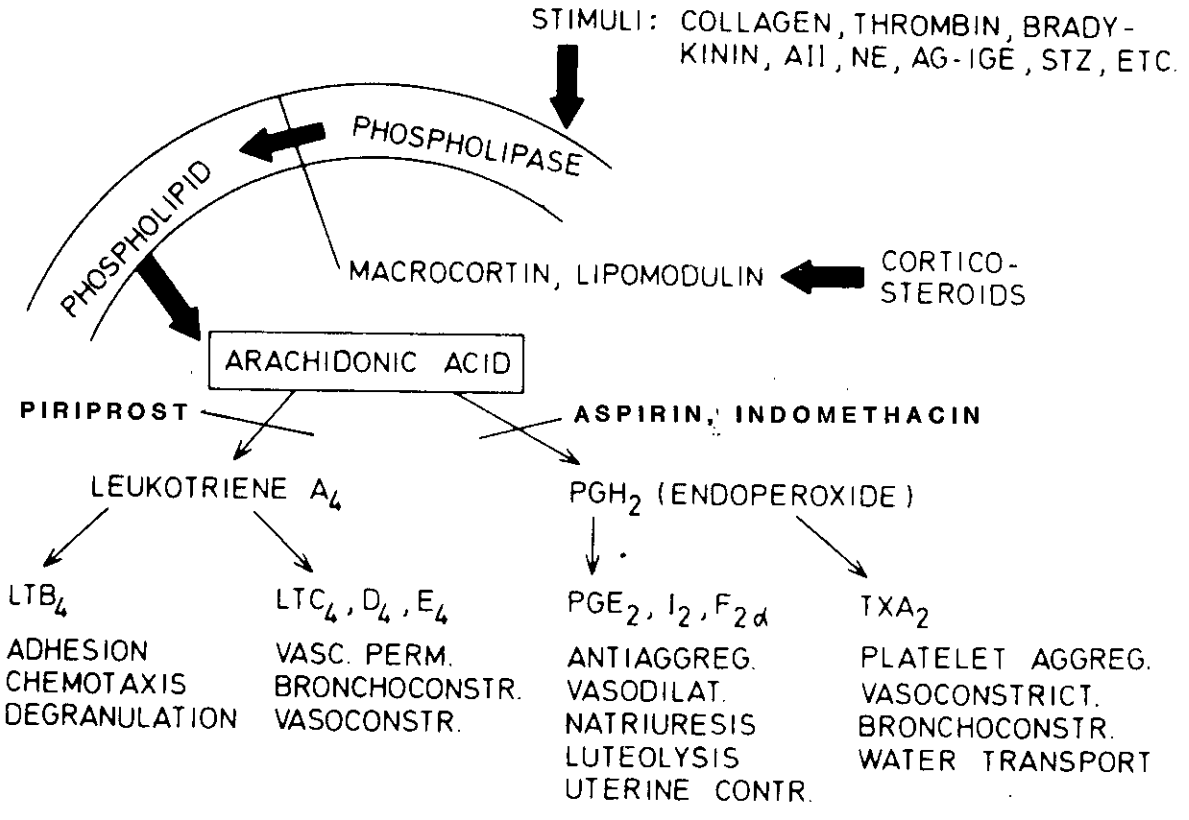
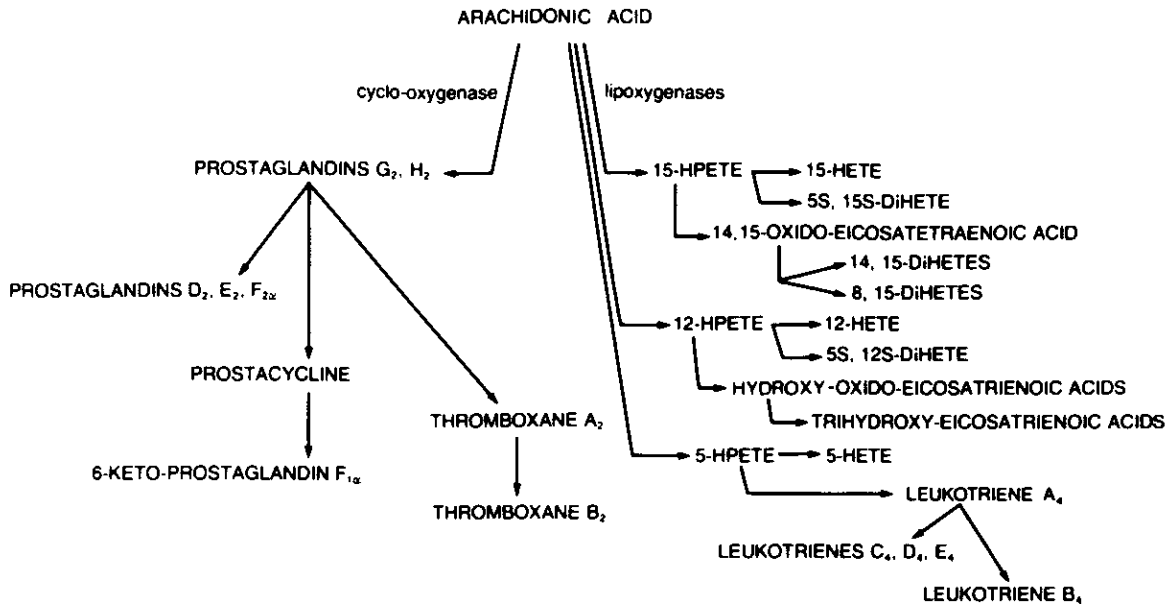
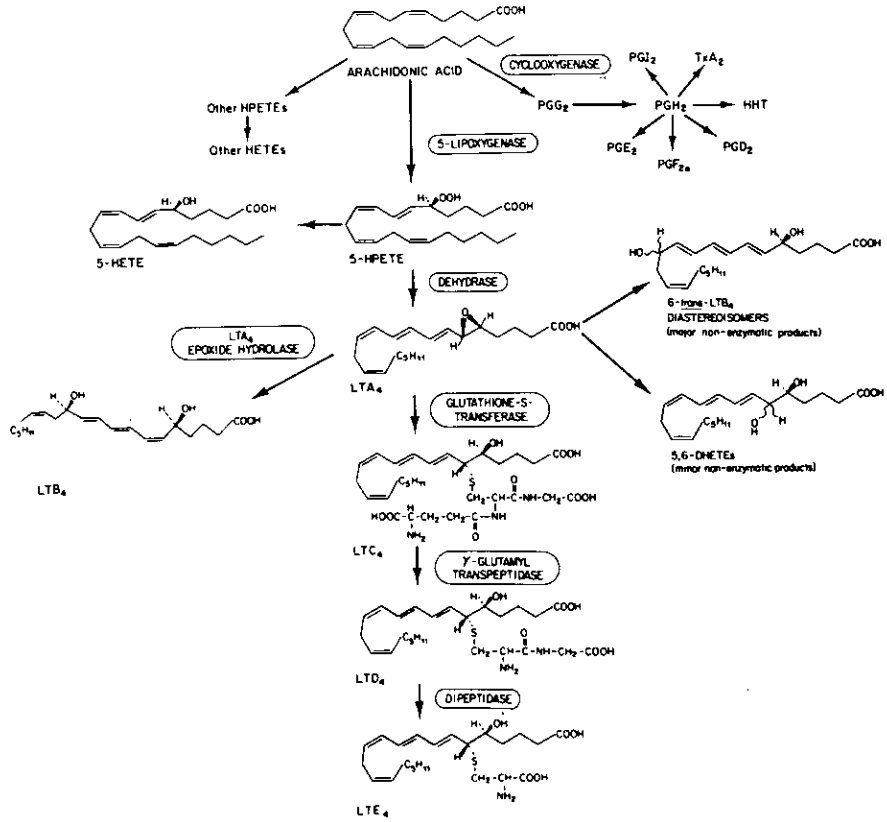

STIMULI: COLLAGEN, THROMBIN, BRADY-
KININ, AII, NE, AG-IGE, STZ, ETC.
PHOSPHOLIPASE
PHOSPHOLIPID
MACROCORTIN, LIPOMODULIN
CORTICO-
STEROIDS
ARACHIDONIC ACID
PIRIPROST
ASPIRIN, INDOMETHACIN
LEUKOTRIENE A_4
PGH_2 (ENDOPEROXIDE)
LTB_4
ADHESION
CHEMOTAXIS
DEGRANULATION
LTC_4, D_4, E_4
VASC. PERM.
BRONCHOCONSTR.
VASOCONSTR.
$PGE_2, I_2, F_{2\alpha}$
ANTIAGGREG.
VASODILAT.
NATRIURESIS
LUTEOLYSIS
UTERINE CONTR.
TXA_2
PLATELET AGGREG.
VASOCONSTRICT.
BRONCHOCONSTR.
WATER TRANSPORT

Figure 2 Top: General scheme of the metabolism of arachidonic acid by cyclooxygenase and the lipoxygenases. (Pierre Borgeat et al 1985)

Bottom: Biosynthetic pathways of leukotriene generation: enzymatic cascade for the oxidative metabolism of arachidonic acid. The enzymes of the 5-lipoxygenase pathway are specifically indicated. (Robert A. Lewis 1984)


ARACHIDONIC ACID
cyclo-oxygenase
lipoxygenases
PROSTAGLANDINS G2, H2
PROSTAGLANDINS D2, E2, F2α
PROSTACYCLINE
6-KETO-PROSTAGLANDIN F1α
THROMBOXANE A2
THROMBOXANE B2
15-HPETE
15-HETE
5S, 15S-DiHETE
14,15-OXIDO-EICOSATETRAENOIC ACID
14, 15-DiHETES
8, 15-DiHETES
12-HPETE
12-HETE
5S, 12S-DiHETE
HYDROXY-OXIDO-EICOSATRIENOIC ACIDS
TRIHYDROXY-EICOSATRIENOIC ACIDS
5-HPETE
5-HETE
LEUKOTRIENE A4
LEUKOTRIENES C4, D4, E4
LEUKOTRIENE B4


ARACHIDONIC ACID
CYCLOOXYGENASE
Other HPETEs
Other HETEs
PGG2
PGH2
PGI2
TxA2
HHT
PGE2
PGD2
PGF2α
5-LIPOXYGENASE
5-HETE
5-HPETE
DEHYDRASE
LTA4
LTA4 EPOXIDE HYDROLASE
LTB4
GLUTATHIONE-S-TRANSFERASE
LTC4
γ-GLUTAMYL TRANSPEPTIDASE
LTD4
DIPEPTIDASE
LTE4
6-trans-LTB4
DIASTEREOISOMERS
(major non-enzymatic products)
5,6-DiHETEs
(minor non-enzymatic products)

arachidonic acid to 15-,12-,and 5-HPETE routes. The last includes slow-reacting substance of anaphylaxis (SRS-A). Borgeat and Samuelsson in 1979 discovered the chemical structure of SRS which consisted of peptidolipids that are derived from 5-lipoxygenase pathway (Fig. 2). These peptidolipids were first discovered in preparations of leukocytes. The term leukotrienes (LTs) was then chosen because the compounds have a common structure feature, "conjugated triene." LTs may have some importance in bone remodeling because of their role as mediators of inflammation, just like that of PGs. The lipoxygenase pathway can be summarized as following: arachidonic acid is converted into an unstable epoxide intermediate 5-HPETE which then dehydrolyzes to form another unstable LTA_4. Enzymatic hydrolysis of LTA_4 produces LTB_4. LTA_4 can also be converted to LTC_4 by adding glutathione. By eliminating glutamic acid with γ-glutamyl transpeptidase, LTC_4 is metabolized to form LTD_4. Cleavage of the glycine portion from LTD_4 produces LTE_4, and enzymatic glutamylation of LTE_4 produces LTF_4. The SRS-A fraction that causes long contraction of guinea pig jejunum is believed to consist of mainly LTD_4, but LTC_4 and LTE_4 are also its components.

The principle biological activities of LTB_4 in vitro are on leukocyte aggregation and movement. LTB_4, either in single doses or at concentrations within the range of

those reported to occur in human synovial fluid, excerts a range of cytotactic effects in vivo. These effects comprise the accumulation of neutrophils at local sites of administration, neutropenia, adherence to and diapedesis of leukocytes from small blood vessels, and increased vascular permeabliity in the presence of vasodilatory prostaglandins. (Smith ~~MJH,~~ 1982) LTB_4 and the PGs may work synergistically to increase vascular permeability. LTC_4, LTD_4 and LTE_4 produce vasoconstriction of small arterioles and increase the permeability of capillaries and venules. They also cause vascular permeability at much lower concentrations than histamine. Dahlen et al (1983) found that LTC_4 was approximately 5000 times more potent than histamine in vascular permeability. An interesting role of LTC_4 is its asthmatic effect on air way smooth muscle. Studies with bronchi from atopic patients sensitive to birch pollen demonstrate the relative importance of leukotrienes as mediator of anaphylaxis. Cysteinyl - containing leukotrienes, LTC_4 and especially LTD_4, have also recently been shown to induce mucus secretion by tracheal submucosal glands in dogs (Johnson ~~H.G.~~ 1982).

It is possible to find some kinds of drugs to inhibit 5-lipoxygenase pathway and to alleviate asthmatic symptoms. U-60,257 (piriprost) is one of those inhibitors (Bach et al 1982. 1984. 1985). Mode of action

agent toward PMN, eosinophiles, and mononuclear cells. Tinker et al (1982) and Burger et al (1984) have demonstrated that monocytes and mononuclear phagocytes are most likely the precursors of the osteoclast. Combination of the above observations implies the possible contribution of LTB_4 towards osteoclastic formation. (3) Increasing vasopermeability and vasoconstrictive ability of LTs as mentioned previous in this text.

The interest in the effect of piriprost to modify resorptive activity and to stimulate or induce formative activity (Collins 1986) are three folds: (1) Since the animals used in that experiment are young, the sutures are undergoing rapid growth remodeling and thus the state of the suture at the beginning of experiment is supposedly formative dominant. When piriprost is used to suppress leukotrienes, the loss of leukotrienes may contribute to the inability of formation of osteoclasts in a compressive environment. And ~~got~~ by certain reasons, eg., low tissue content of leukotrienes favors formation of osteoblast and thus enhances the formative activity which might have been present in the suture before the injection of piriprost. (2) Piriprost may also inhibit formation of osteoclasts from monocytes or inhibits migration of monocytes to the experimental suture. (3) Piriprost directly or indirectly stimulates formation of

osteoblasts. Besides the above possibilities, there are two other points: a, Although piriprost modifies the histological picture, the osteoclastic activity must have been previously present, because there was also evidence of increased marrow spaces and lacunae in the pressure group rather than simple deposition. b, The osteotomized sutures may be different from normal sutures and may not be representative of normal state of the sutures, but are necessary controls to relieve endogenous stress.

The author would also like to stress the importance of synchrony of animal biological rhythms, especially for hormonal or metabolic studies. The classic concept was that synchrony was conferred or adjusted by the duration of the environmental light period. More recently, it has been realized that meal timing can also have a significant and often dominating effect (Nelson et al 1975; Simmons 1979) on the biological rhythms. "Biological studies that are in essence spot checks of cell activity can be adequately controlled by conducting all work at precisely selected time of the body rhythm" (Simmons 1979). Because of the periodical biologic function is dependent upon time of day (circadian rhythm) and duration of experimental period, the findings may also be dependent on the experimental design in terms of timing.

In the study Collins, he found only slight PGE_2

concentration elevation in pressure group in comparison to control. But the study of Radulovich showed almost four times PGE_2 elevation in comparison to control. This difference might be because of different sacrifice time. Collins used noon time while Radulovich used evening time. It is interesting to see what kinetic changes of leukotrienes are in other time periods, for example: in the initial stage of applying force.

No experiment was ever done to inhibit both cyclooxygenase pathway and 5-lipoxygenase pathway of cellular metabolism in the rat sutural areas. It will be interesting to evaluate the cellular behavior with low or no presence of the two important arachidonic metabolites because double inhibition of both pathways may cancel the formative remodeling activity found in Collins study.

STATEMENT OF THESIS

In order to evaluate the cellular behavior and metabolic content in other time period than those of Collins' (1986) under the influence of piriprost and compressive force to the midsagittal sutre of rats, the experimental time was lenghtened to six days and the drug administration was started from the fourth day through the end of experiment. Therefore the resorptive response could first be induced before piriprost was administered so as to allow evaluation of the ability of piriprost to induce osteoblasts or to stop osteoclastic activity.

Total of thirty-six growing female Sprague-Dawley rats were used and divided into 6 groups. The design of different treatment to each group was made to be able to detecte the possible significant effect of compressive force and influence of piriprost to the sutural system. In addition to piriprost, indomethacin was also administered to inhibit biosynthesis of both LTs and PGE_2, in one of the experiemental groups.

The 20 g compressive force was delivered by surgically implanted spring cross the midsagittal suture. The suture area was removed and examined histologically and biochemically before and after the metabolites were depressed by piriprost administration. Comparative descriptive histological data was collected to analyze along with the biochemical data within each group. The

results of these experiments were also compared with those of Collins (1986) and of Radulovich (1985) on comparable basis.

Experimental Design

The design of the method and materials is made to follow the protocol of Collins (1980) as close as possible (Table I), so that the result will be on a comparable basis. Some major differences among my designs and those of Collins and of Radulovich are listed in Table X.

The 36 female Sprague-Dawley rats were divided into 6 groups with 6 in each group (Table I). At the start of the experiment period, they were 21 days old and were sacrificed 6 days later (Group C were sacrificed 3 days later). The 6-day length experiment was to allow first 3-day non drug and later followed by a 3-day drug treatment. This design was different from that of Collins and was to allow possible stimulation of resorption in the sutural system during the first 3 day compressive treatment and then followed by 3-day drug reversive action. Group A, B, C, and D were control groups. Group A was treated only with osteotomy, no pressure, no drug applied. Group B were treated the same as Group A, but piriprost (Upjohn Co., Kalamazoo, MI) was injected for the last 3 days of experiment. Group C received osteotomy and 20 gms of compressive force and were sacrificed on the 4th day. Group D were treated the same as Group C except that they were sacrificed on the 6th day. Group E and F were experimental group. The experimental times for both group were 6 days. Group E

received 20 g of pressure and piriprost which was injected only for the last 3 days. Group F received every thing that Group E did but indomethacin which was also injected simultaneously when the piriprost was injected.

TABLE I

EXPERIMENTAL DESIGN

EXPERIMENTAL CONDITION EXPERIMENTAL CONDITION	GROUP	BIOCHEMICAL ANALYSIS # OF ANIMAL	HISTOLOGICAL EVALUATION # OF ANIMAL
CONTROL (6 DAYS) (OSTEOTOMY ONLY)	A	4	2
CONTROL (6 DAYS) + PIRIPROST FOR THE LAST 3 DAYS	B	4	2
PRESSURE FOR 3 DAYS	C	4	2
PRESSURE FOR 6 DAYS	D	4	2
PRESSURE FOR 6 DAYS + PIRIPROST FOR THE LAST 3 DAYS	E	4	2
PRESSURE FOR 6 DAYS + PIRIPROST AND INDOMETHACIN FOR THE LAST 3 DAYS	F	4	2

Methods and Materials

Animals

This study used 36 female Sprague-Dawley rats. They were delivered at 16 days of age from Zivic Miller Laboratories (Allison Park, PA). For consistency of growth the animals were special ordered to weight approximately 30-35 gms on delivery. Five days between delivery and the start of the project allowed the animals to recover from stress, from the delivery and to adjust to their new environment. The pups were force-weaned at 19 days of age to prepare them for independency from the mother at the beginning of the experiment (21 days).

The beginning of the experiment for the rats started at age of 21 days which was exactly the same used by Collins. Their weight at this time ranged from 43 gms to 65 gms. Care was taken to attempt maintaining a constant circadian rhythm of the animals with a consistant light-dark cycle (6:45 a.m. - 6:45 p.m.) and minimal disturbance of the pups during the experimental period. The animals were weighed daily just prior to dark cycle from the first day of delivery through the end of the experiment. The bedding (Wood Particles1, American Excelsior Co., Arlington, TX) was changed every 3 days. The animals were fed a diet of Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and tap water ad libitum. Each cage (17"x13"x8") housed one group of six animals in approximately 24°C and 10-20% humidity controlled animal room.

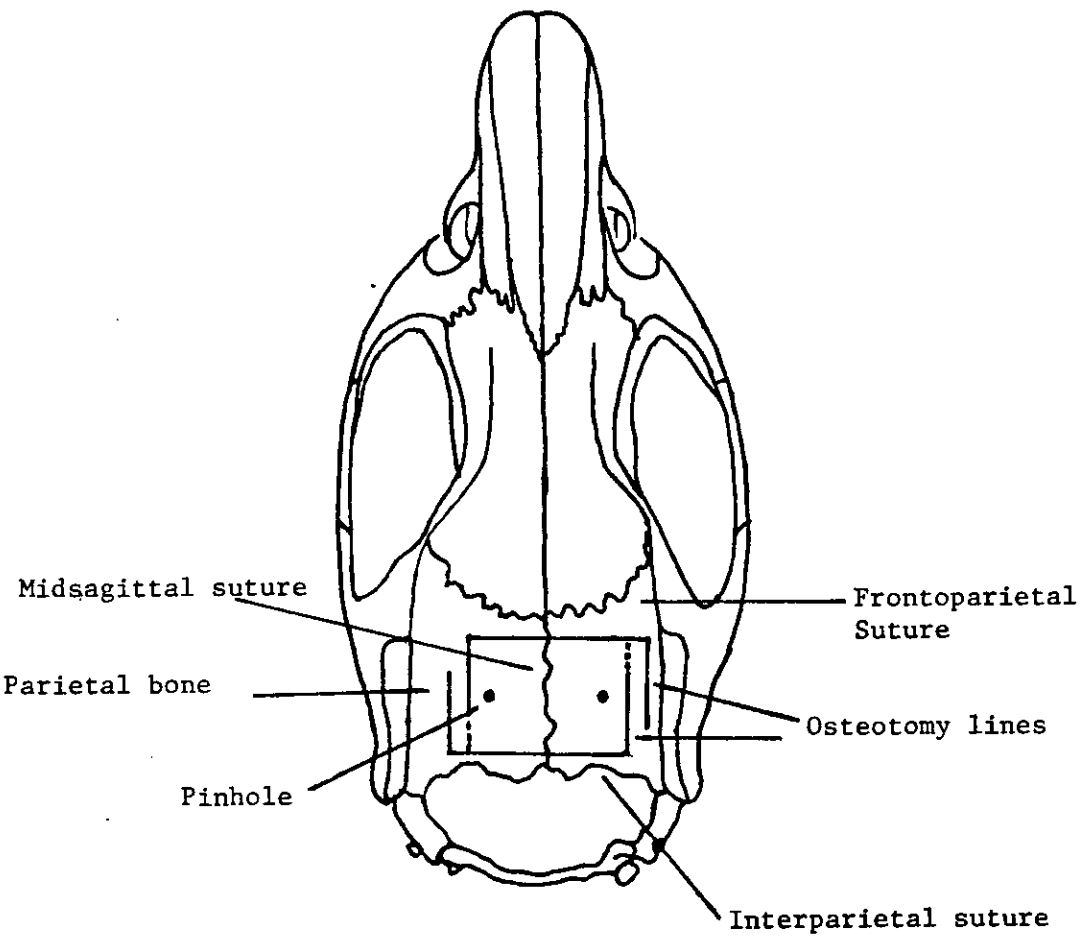
The injections started at the beginning of 4th day of experiment and were given repeatedly immediately before and after the dark cycle of each 24 hr period through the last 3 days of the experimental period. The dosage for piriprost was 10 mg/kg and for indomethacin, 1 mg/kg body weight. The animals were weighed daily and the amount of drug administration was calculated and adjusted accordingly.

These animals underwent surgical procedures consisting of calvarial osteotomies in the parietal bones (Figure 3). Following osteotomies a compressive coil spring was implanted in the parietal bones of animals in Group C, D, E, and F. Before seating of each spring, the two legs of the spring were adjusted to deliver 20 g of compressive force to the two holes drilled 5 mm apart on the parietal bones across the midsagittal suture. The design of the osteotomy allows the bone segment to maintain continuity with the surrounding structures, yet produces a segment capable of isolating compressive force.

The described grouping of the animals made it possible to evaluate the different effects of the 20 gm compressive force, leukotriene inhibitor and indomethacin. The combined effect of piriprost plus indomethacin on the sutures has never been performed before. The different treatment timing of piriprost and

Figure 3 Diagram of Osteotomy and Sample Removal

Diagram shows cutting lines of osteotomy on the parietal bones. The two pinholes were drilled 5 mm away from each other in a 90-degree angle across the midsagittal suture. When the sutural sample is to be removed, simply connecting the dotted lines will make it.


Midsagittal suture
Frontoparietal Suture
Parietal bone
Osteotomy lines
Pinhole
Interparietal suture

indomethacin from those of Collins and of Radulovich made it possible to reveal the possible reverse effect of piriprost on resorptive action induced by compressive force. The metabolite levels were obtained by protein assay and radiommunoassay of LTB_4, LTC_4 and PGE_2.

At end of the experimental period, 4 animals from each group were randomly chosen for biochemical study and sacrificed to remove the bone segments. Granulation tissue and blood products surrounding the bone segment were gently scraped and rinsed with normal saline, the edges of the osteotomy were also scraped to eliminate any possible changes of the biochemical mediators from fracture healing.

The bone specimens were then quick frozen in liquid nitrogen to preserve and minimize biochemical changes at the time of removal. The samples were lyophilized overnight (~ 12 hours) and then stored in a freezer at -80°C for future extraction and analysis for LTB_4, LTC_4, PGE_2 and protein assay.

Two other randomly chosen animals were perfused using 1/2 strength Karnovsky solution. Once perfusion was complete the osteotomy was removed for light microscopy. A segment of bone sample located at the side of osteotomy including the cutting wound was also removed to be examined for the healing process in other project.

The sutural samples for the histologic study were

placed in series of fixative procedures including Millonig solution, osmium tetraxide, uranyl acetate, alcohol series and propylene oxide/maraglass mixture for maraglass embedding. Histological descriptive analysis was performed to yield corollary histologic evidence with the biochemical analysis.

Appliance Construction

The appliances used to deliver the compressive forces were designed and used by Benson (1983). Radulovich (1985) and Collins (1986) used similar appliances. They are constructed from 0.014 inch round austenitic orthodontic stainless steel wire (Unitek Corporation, Monrovia, CA).

All springs were fabricated by first placing a single coil with a 0.5 mm extension in the wire. This was accomplished by wrapping the wire around the small barrel of the Tweed loop-forming plier (Unitek Corporation). These extensions were used to anchor the spring to the calvaria by placing them in bilateral pinholes drilled in the osteotomy (Figure 3). Further retention was maintained by bending the extentions inward approximately 60° from the horizontal. The single coil on the legs was included to prevent excessive penetration of the wire into the underlying tissues.

The next step in the fabrication was to make two 7 mm arms separated by a multihelical coil. This coil was formed by placing four loops of wire around the large barrel of a Tweed loop forming plier. This coil provided the flexibility in the spring to elicit a reasonable force of 20 g.

All springs were preadjusted with a precision force gauge (Haldex, Halmstad, Sweden) to produce 20 g of force when expanded to 5 mm. Placement of springs and final calibration is discussed in the surgical section.

Anesthesia

A. Surgical procedure Anesthesia

Induction of anesthesia for implant surgery was produced by an intramuscular gluteal injection of approximately 0.1 ml 90% Ketamine hydrochloride solution, (100 mg/ml Ketaset, Bristol;) and 10% Acepromazine Maleate (10 mg/ml Ayerst Lab. Inc.). These drugs were used in combination to facilitate handling of the animal and to increase the margin of safety over and above the sole use of a single drug. The Ketaset was used to produce anesthesia which causes the animal to be immobile and free of pain. The Acepromazine was used as a tranquilizer which renders the animal relaxed. In addition to these two drugs approximately 0.1 ml local subcutaneous infiltration injection of 2% Xylocaine

hydrochloride with epinephrine 1:100,000 (Astra Pharmaceutical Products Inc., Worcester, MA) was given. This reduced sensation in the surgical area and prevented any sudden jerks during the surgical procedures.

B. Sacrifice Anesthesia

The animals in both biochemical assay groups and histologic evaluation groups were given an approximately 0.13 ml intramuscular injection of 90% Ketamine HCl prior to sacrifice. No lidocaine was used for sacrifice because it might have an adverse effect on the tissue metabolite content. After the animals were immobilized by Ketamine, an approxiamtely 0.13 ml of sodium pentobarbital (60 mg/ml Diabutal, Diamond Lab, Des Moines, IA) was injected intraperitoneally to put the animal in deep anesthesia before sacrificing. If the amount of sodium pentobarbital had not been enough, an increments of 0.05 ml was injected until the animal no longer responded to tail pinching. The osteotomy was then removed immediately after the animal had sound anesthesia.

Surgical Procedures

The purpose of this procedure was to isolate sutural segments and to implant coil springs that was applying 20 g compressive force across the midsagittal sutures.

Once anesthesia was induced, the animals were weighed and prepared for surgery. Using a pair of scissors, the hair on the top of the head was cut in an area extending from the back of the neck to between the eyes. This area was swabbed with copious amount of Povidone-iodine (Betadine, Butler Co., Warren, OH) and blotted dry with a sterile 2"x2" gauze. An incision was made with a scapel in the midline from just behind the eyes extending a few millimeters behind the ears. The periosteal tissue covering the osteotomy was carefully deflected with normal saline soaked cotton tip applicator. This method was proved to be less traumatic and was causing less bleeding. The calvaria was rinsed with normal saline (0.9% Nacl, pH 5.7, Butler Co., Warren, OH) and blotted dry with a sterile 2"x2" gauze.

To obtain congruent osteotomies in all animals an acrylic template made from Orthoacryl (Stratford-Cookson Co., College Park, GA) was constructed (See Collins 1986). It contained the outlines of the osteotomy including the two pinholes for anchorage of the spring. While tracing the osteotomy outlines on the skull, the template was aligned with the midsagittal suture and placed midway between frontoparietal and the interparietal suture and then tracing was done by razor point felt tip marker. The two pinholes were exactly 2.5 mm on either side of the midsagittal suture, thus 5 mm of

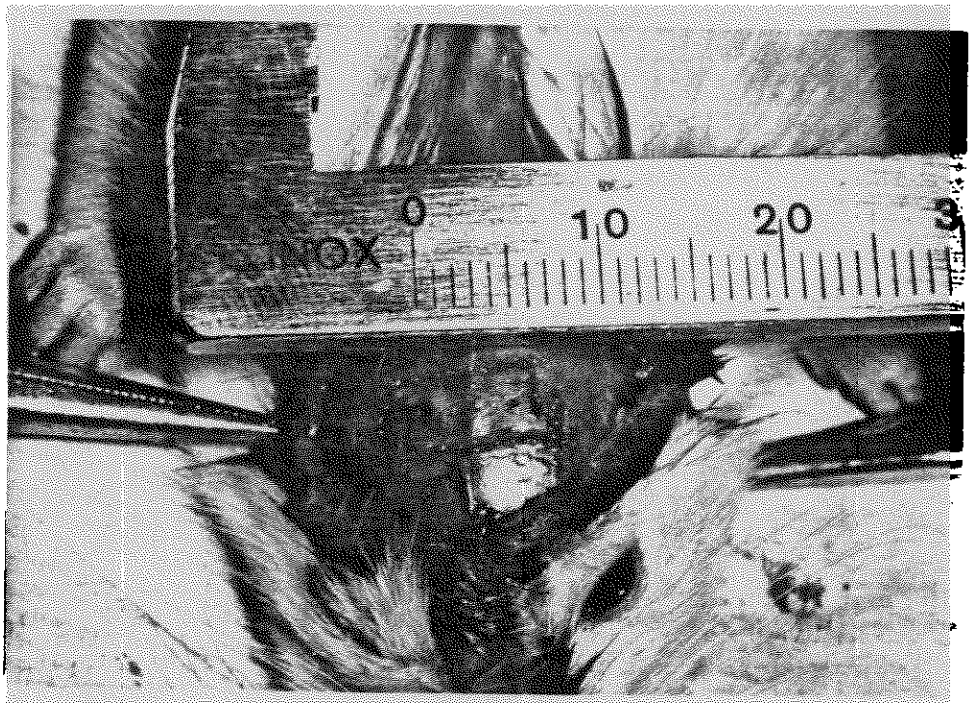
separation existed between the holes.

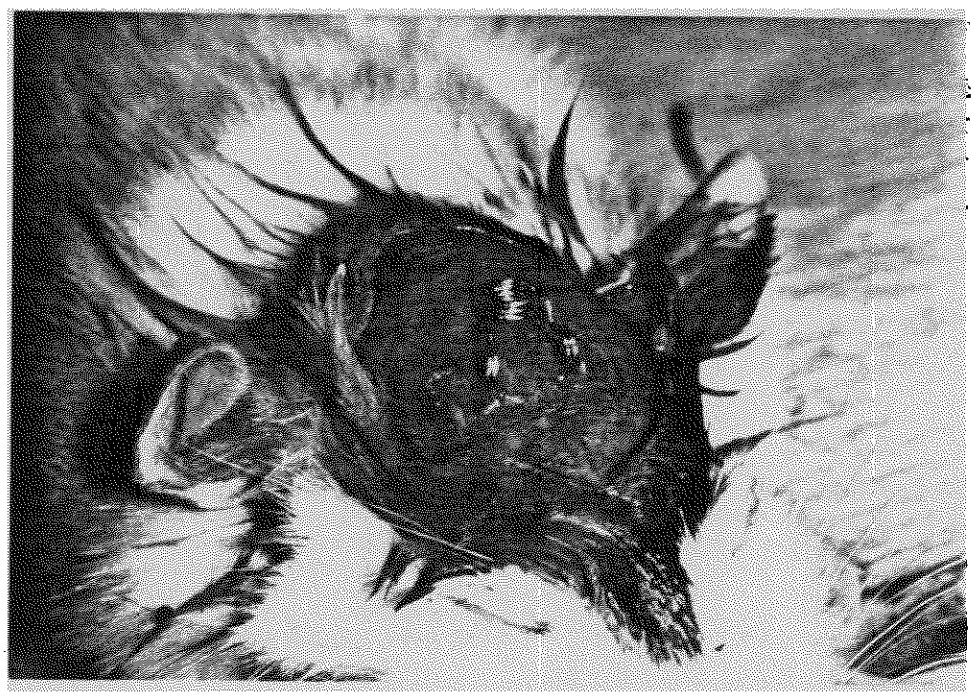
The osteotomy was drilled and cut into the bone utilizing #1/4 round carbide bur in a slow speed dental engine. The cuts were made completely through the bone, however, special care was taken not to injure the underlying dura matter or to puncture the sagittal sinus. While drilling the osteotomy the bone was irrigated frequently and thoroughly with saline solution. This was to prevent the bone tissue from over heating due to friction of the cutting instrument and to remove any pulverized bone tissue which might initiate or accelarte fracture healing (Plate I).

When the osteotomies were completed (~20 min. for each animal), all animals except group A and B had implanted in them with a compressive spring. All springs were adjusted to deliver 20 g of force by inserting one wire leg of the spring into a pinhole while the other leg was placed into the opposite hole with the precision force gauge. All springs were checked two to three times and readjusted if necessary before cementing into the pinholes to make sure that each one of them was delivering 20 g of compressive force. Once the springs were in place, a small drop of cyanoacrylate cement (Super Glue Co., Hollis, NY) was applied with a dental explorer around the hole to retain the spring. After 30 seconds the bond strength was tested by placing a dental

Plate I The pinholes and osteotomy drilled by slow speed dental engine on the parietal bones of the calvaria. The distance between the two pinholes is 5 mm. The bluish bulging center zone is midsagittal suture.

Plate II Placement of the compressive spring on the calvaria with superglue to ensure the stability of the spring legs in the pinholes.


0
10
20



and light. Therefore in preparation of the drug care was taken to work quickly and in an ice bath under dark cover when possible. Because the drug is soluble in water it was diluted with normal saline to a final concentration of 3.5 mg/ml solution for injection. Since the free acid tends to come out of solution when the pH drops below 6.5, the pH was adjusted utilizing a micropipet and pH paper (Short Range Alkacid 6.0-8.8, Fisher Scientific) to a pH of 7.0-7.2 with saturated K_2CO_3 and 2 N HCl.

The drug was stored in 1.5 ml snap-cap polypropylene vials (39 x 10 mm, Sarstedt, W. Germany) at -80°C, a temperature at which the drug is reasonably stable. Only enough drug for a series of injections was removed from the freezer. The piriprost was thawed, inspected for solubility and injected at a dose of 10 mg/kg twice daily. The injections were given at the beginning and at the end of the light cycle of each 24 hour period 8:00 a.m. & 6:00 p.m. for the designated groups and days. The animals were weighed prior to the beginning of the dark cycle and the amount of drug administered was adjusted to maintain a dosage of 10 mg/kg (0.15 ml - 0.24 ml per injection). Subcutaneous injections were given after loosening the skin over the suture by lifting the skin of the neck right behind the ears and administering quickly. The drug appeared to have no deleterious effects on the

animals (see Collins toxic test 1986). All pups appeared healthy and normal throughout the experiment.

Indomethacin (Indocin, Tarr Industrial Drug Co., Cleveland, OH) was chosen to treat only group F together with piriprost. It is a potent inhibitor of prostaglandin synthesis. Because indomethacin is insoluble in water, aqueous suspensions were prepared for administration. To prepare the suspensions, the contents of the capsules were opened and weighed to 25 mg. The 25 mg of powder was then placed in 125 ml flask on a magnetic stirrer and diluted with 62.5 ml of normal saline to make 0.4 mg indomethacin/ml suspension. During mixing with the magnetic stirrer, the suspension was dispensed into 6 seven-ml scintillation vials and was stored at -80°C ready for group F 3 days morning and evening injections.

Indomethacin injections were given only to group F starting from the beginning of 4th day through the end of experiment together with piriprost injections. Each rat was weighed at the p.m. injection time and the dosage was adjusted accordingly to keep it at 1 mg/kg. In order to minimize the injections and stress to the experimental rats, appropriate amounts of piriprost and indomethacin were mixed together right before each injection to each animal.

Sacrifice of the animals

A. Biochemical part sacrifice

Sacrifice of the animals for biochemical assays took place between 8:00 p.m. and 9:00 p.m. on the third day (78 hours after operation) of the experiment for group C and on the sixth day (150 hours after operation) for all other groups. The animals were weighed and then given a 0.13 ml intramuscular gluteal injection of Ketamine/Acepromazine solution. The surgical area was reopened and the springs were checked for correct and unaltered position before removing. The removed spring was checked to evaluate its remaining compressive force.

Granulation tissue covering the osteotomy was removed with the #15 scalpel. With a #12B scalpel and a dental explorer (American Dental U.S.A., ZM0320, AMFLEX II) the osteotomy cuts were connected and detached from the underlying connective tissue (Figure 3). Once the bone specimens were removed they were irrigated thoroughly and rapidly with normal saline to remove blood products. The bone was gently scraped with the #15 blade to remove excess granulation tissue. The edges of the bone were scraped to remove any cells accumulated in response to fracture healing caused by the osteotomy cuts. After quickly cleaning up the blood clots and bone edges, the samples were then transfered to a 12 mm x 75 mm Pyrex culture tube (Corning Glass Works, Corning, NY)

which had previously been cooled and filled with liquid nitrogen in an ice bucket. When all samples within each group were collected, they were immediately placed in a 300 ml fast freeze flask (Labconco, Fisher Scientific, Pittsburgh, PA) along with a dye indicator tube and lyophilized for 11-12 hours (Virtis, Unitrap Model 10-100, Vistar Co., Gardiner, NY). At the end of the lyophilization the samples were stored at -80°C in the Pyrex Culture tubes which were sealed with Parafilm (American Can Co., Greenwhich, CT) until ready for pulverization, extraction and assaying.

B. Histological Evaluation Sacrifice

The animals for histological evaluation were sacrificed between 3:00 and 4:00 p.m. on the third day (~72 hrs) of the experiment for group C and on the sixth day (~144 hrs) for all other groups. This different timing from biochemical sacrifice is because it is impossible to sacrifice all animals at a time. The animals were weighed and then given a 0.2ml intramuscular gluteal injection of Ketamine/Acepromazone mixed sol'n. An intraperitoneal injection of 0.15 ml Sodium pentabarbital was given to deep anesthesize the animal. If necessary 0.05 ml of increments was given till the animal did not respond to tail pinches. The skin of the chest wall was incised and deflected. The rib cage was

cut and reflected up to expose the heart. A 21-gauge hyperdermic needle was inserted into the left ventricle while the perfusion pump (Masterflex, Cole Palmer, Chicago, IL) was pumping the one half strength Karnovsky fixative through the needle. Right after inserting the needle into the heart a small cut in the right auricle was made to allow for exit of the blood. The total perfusion time was about 4 minutes.

The osteotomy area was reopened around the spring area with scissors so that the seating of the spring would not be disturbed before checking its position. Soon after the wound was reopened, the osteotomy area was rinsed with half strength Karnovsky solution to keep it wet with the fixative. The specimen was then removed in a similar way as that described for the biochemical assay and put into half strength Karnovsky solution. After 2 hours of washing with half-strength Karnovsky the sample was taken through a series of solutions consisting of Millonig's buffer, 4% osmium tetroxide buffer, distilled H_2O, 0.25% uranyl acetate, 50% , 70%, 95% and 100% ethyle alcohol series, propylene oxide, 1:1 propylene oxide/maraglass epon mixture and eventually embedded in maraglass/epon mixture. The specimens were then sectioned at 1μ on a Ultratome (Ultratome III, Type 8802, LKB-Produkter, AB Stockholm-Bromma-1, Sweden). The sections were stained with 0.3% toluidine blue 0 (Fisher,

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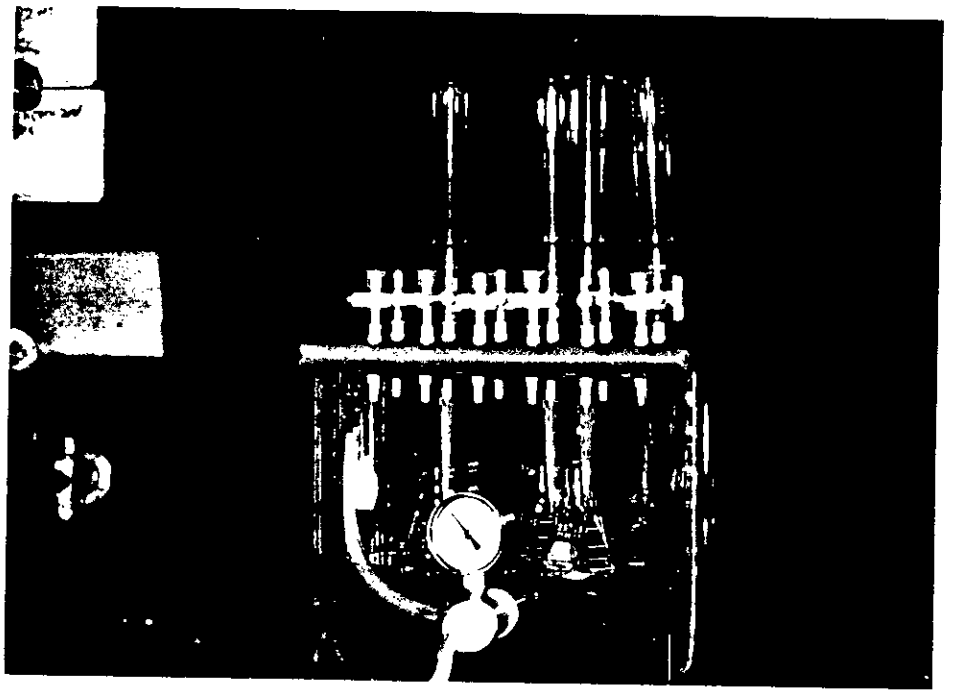
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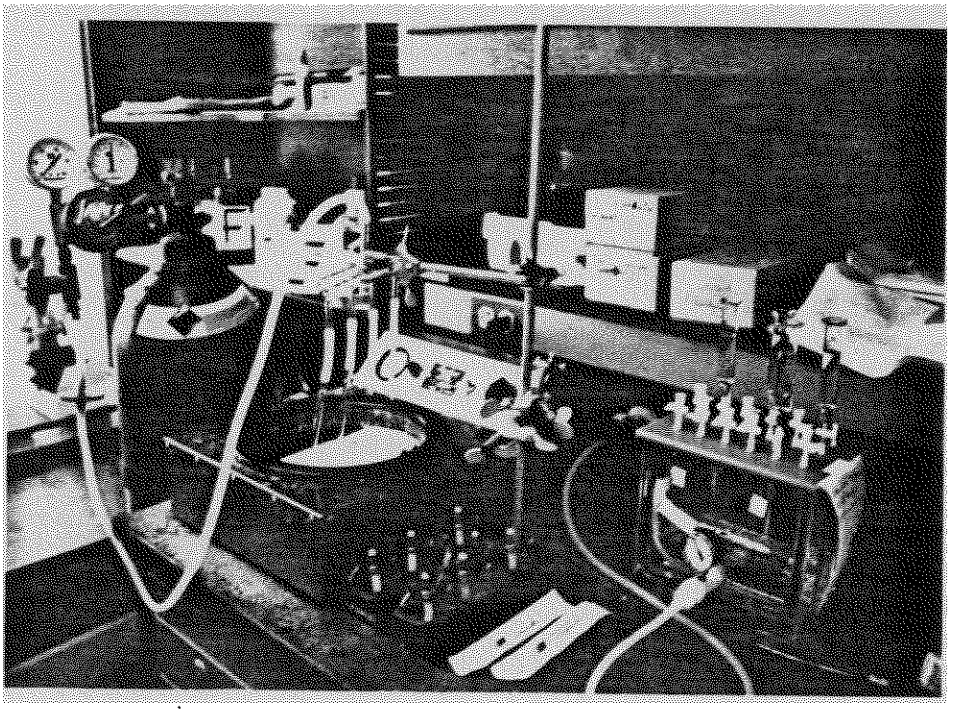
Plate III Extraction Apparatus.

Alltech Vacuum Manifold with glass syringes attached to the outer inlets and C-18 Microclean-up separating columns attached to the inner inlets. Inside of the manifold, ice bath and flasks or test tubes can be set up to collect the eluted extract. When the vacuum manifold was operating, the negative pressure was close to -80 mm Hg.

Plate IV Evaporation Apparatus and Alltech Vacuum Manifold.

Evaporation Apparatus includes N_2 gas tank, siliconized tubing, gas filter, gassing manifold Pasteur pipetts, extract in flasks, 30°C water bath, ring stand with clamp and holders.





environment to minimize loss of leukotrienes by the light. Cold (0-4°C) 1 M perchloric acid (PCA) was added to the bone samples in a ratio of 10 mg bone/1.0 ml PCA and allowed to stand with cover in an ice bath for 20 minutes with occasional gentle mixing.

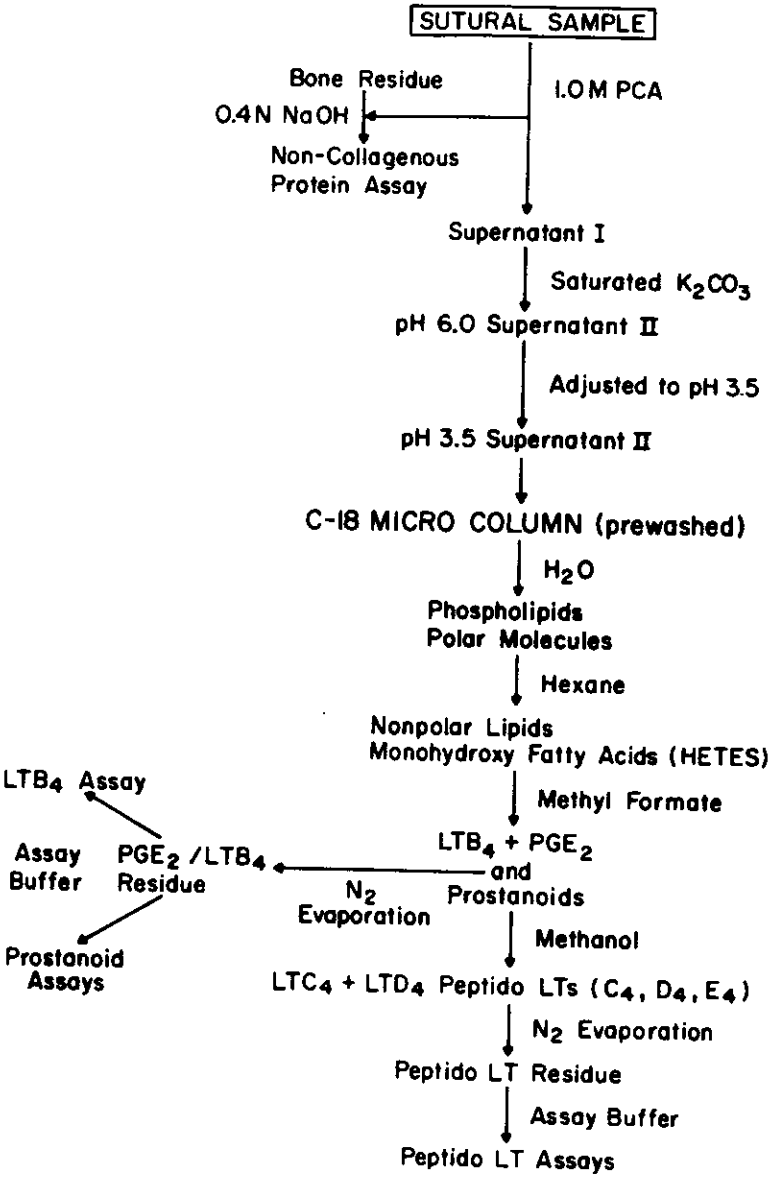
The PCA treated samples were spun with an automatic refrigerated centrifuge (Sorvall Superspeed, RC 2-B, Ivan Sorvall Inc., Newton, CT) at 45,900g (19,500 rpm/SM-24 rotor) for 20 minutes at about 3°C. The supernatant were decanted to the other sets of 13 x 100 mm polypropylene centrifuge tubes. The acid insoluble pellets were returned to the -80°C freezer and stored for protein assay.

The supernatants were neutralized with saturated potassium carbonate (K_2CO_3) to pH 6.0 as determined with pH paper (pHydrion 5.2-6.6, Micro Essential Laboratory, Brooklyn, NY). The correct neutralizing volume ratios were determined by titrating each batch of PCA and K_2CO_3 at the time the reagents were prepared. The affinity of leukotrienes for the separation column is greater in the acid form. Therefore, the neutralized solution was back titrated with 2 N hydrochloric acid (HCl) to pH 3.5 as determined with phydrion 3.0-5.5.

The back titrated extracts were centrifuged at 3730 g for 20 minutes. The amount of supernatants were transferred quantitatively by micropipettor to 1.5 ml

Figure 4 Flow chart of bone sample processing.

Starting material is the lyophilized bone sample.


SUTURAL SAMPLE
Bone Residue
0.4N NaOH
Non-Collagenous Protein Assay
1.0M PCA
Supernatant I
Saturated K_2CO_3
pH 6.0 Supernatant II
Adjusted to pH 3.5
pH 3.5 Supernatant II
C-18 MICRO COLUMN (prewashed)
H_2O
Phospholipids
Polar Molecules
Hexane
Nonpolar Lipids
Monohydroxy Fatty Acids (HETES)
Methyl Formate
LTB_4 + PGE_2
and
Prostanoids
N_2 Evaporation
LTB_4 Assay
Assay Buffer
PGE_2 /LTB_4 Residue
Prostanoid Assays
Methanol
LTC_4 + LTD_4 Peptido LTs (C_4, D_4, E_4)
N_2 Evaporation
Peptido LT Residue
Assay Buffer
Peptido LT Assays

the nonpolar lipids and monohydroxy fatty acids are eluted with the addition of 10 ml of hexanes (Certified, Fisher Scientific). After most of the unwanted substances were eluted by water and hexane, PGE_2 and LTB_4 then were collected by eluting with 10 ml of methylformate (Spectrograde, Fisher Scientific) into siliconized 15 ml glass test tubes held inside of the vacuum manifold in an ice bath (0-4°C).

The methylformate fraction was evaporated using a manifold (Plate III) under a stream of purified nitrogen to a volume of 1.0 ml) in a 30°C water bath. The glass test tubes were rinsed twice with 0.5 ml of methylformate and transferred to 4 ml siliconized capped vials (Wheaton Scientific, Millville, NY). This fraction was evaporated to dryness (~15 min.). The dried vials were then quickly sealed with teflon tape, capped, labelled,and stored at 80°C for future LTB_4 and PGE_2 assay at the earliest possible time. Subsequent elution of the column with 10 ml methanol (Spectranalyzed, Certified A.C.S., Fisher) results in a fraction containing LTC_4 and other peptidoleukotrienes. The same procedure of collection and evaporation was applied for the methanol fraction except that the test tubes were rinsed twice woth 0.5 ml of methanol prior to evaporation. The time needed to evaporate methanol was about 3 times longer than that was needed for methylformate.

A preliminary study to test the efficiency of extraction technique and the new C_{18} columns was performed. Recovery concentrations of 1 ng PGE_2 prepared from a 100 ng PGE_2/ml standard (PGE_2 ^{125}I RIA Kit, New England Nuclear, Boston, MA), 8 ng LTC_4 prepared from a 1.6×10^3 ng LTC_4/ml standard (LTC_4 ^{3}H RIA Kit, New England Nuclear) and 668 pg LTB_4 prepared from a 40 ng/ml standard (LTB_4 RIA Kit, New England Nuclear) were added to 650 μl PCA and were processed by the same way as the bone sample extracts.

The same recovery procedures were performed along with each group of experimental samples. The result of the recovery will be included in the result section.

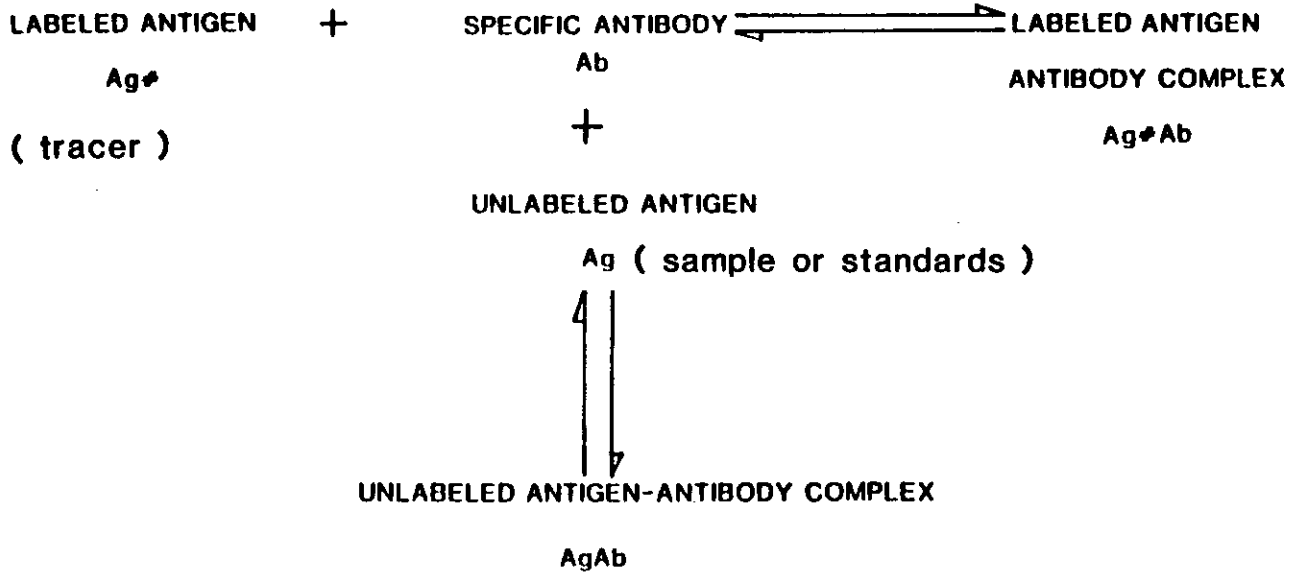
Assay for LTB_4, LTC_4 and PGE_2

The assays used were radioimmunoassays (RIA). the reason for their use was the specificity and sensitivity needed for the assays. The basic principle of RIA depends on competitive antigen binding. Competition occurs between the isotopically labeled antigen (tracer) and unlabeled antigen (standards or samples) for the antibody (antiserum) (Figure 5). When increasing amounts of unlabelled standard or sample are added to the antibody the amount of labelled antigen-antibody complex is reduced. This results in lower radioactive counts.

Figure 5 Schematic of Radioimmunoassay Principle.

The mechanism of this assay technique depends upon competition between fixed number of radioactive antigen (tracer) and unknown number of non-radioactive antigens (samples or standards) for a fixed number of antibody binding sites. The labelled antigen-antibody complex then is detected after certain amount of incubating time to determine the amount of unknown unlabelled antigen.

PRINCIPLES OF RIA



was followed except that the first standard tube was skipped and the centrifugation condition was performed at 3730 g for 30 minutes (see Collins, 1986). The radioactivity was counted on the ^{3}H channel for 2 minutes. A typical LTC_4 standard curve is shown in Figure 7.

LTB_4 Assay

The LTB_4 content was measured by RIA (LTB_4 $^{3}[H]$ RIA Kit NEK-037, NEN, Boston, MA). The methylformate fraction evaporated samples were reconstituted with LTB_4 Kit assay buffer (pH 8.6 Tris./HCl) to an equivalent concentration of 4.5 mg bone/ml assay buffer. The lower concentration of bone in mg per ml was to increase the reconstituted sample amount because methylformate fraction is used for both LTB_4 and PGE_2 assays. The assay technique for LTB_4 is the same as that of LTC_4. The standard curve is shown in Figure 6.

PGE_2 Assay

The PGE_2 content was measured with RIA (PGE_2 $^{125}[I]$ RIA Kit NEK-020, NEN, Boston, MA). Because LTB_4 and PGE_2 eluted in the same fraction and were first reconstituted with LTB_4 Kit buffer (see LTB_4 Assay) I backtritrated all reconstituted samples with 1 N HCl to pH 6.8 as soon as the LTB_4 assay was done and immediately before PGE_2

Figure 6 Standard curve of LTB_4 RIA. Values of LTB_4 for unknown samples were interpolated from a standard curve determined by assaying known quantities of LTB_4 and plotting concentration (picograms LTB_4/tube) versus normalized percent bound (% B/Bo).
Incubation time: 21 hrs.

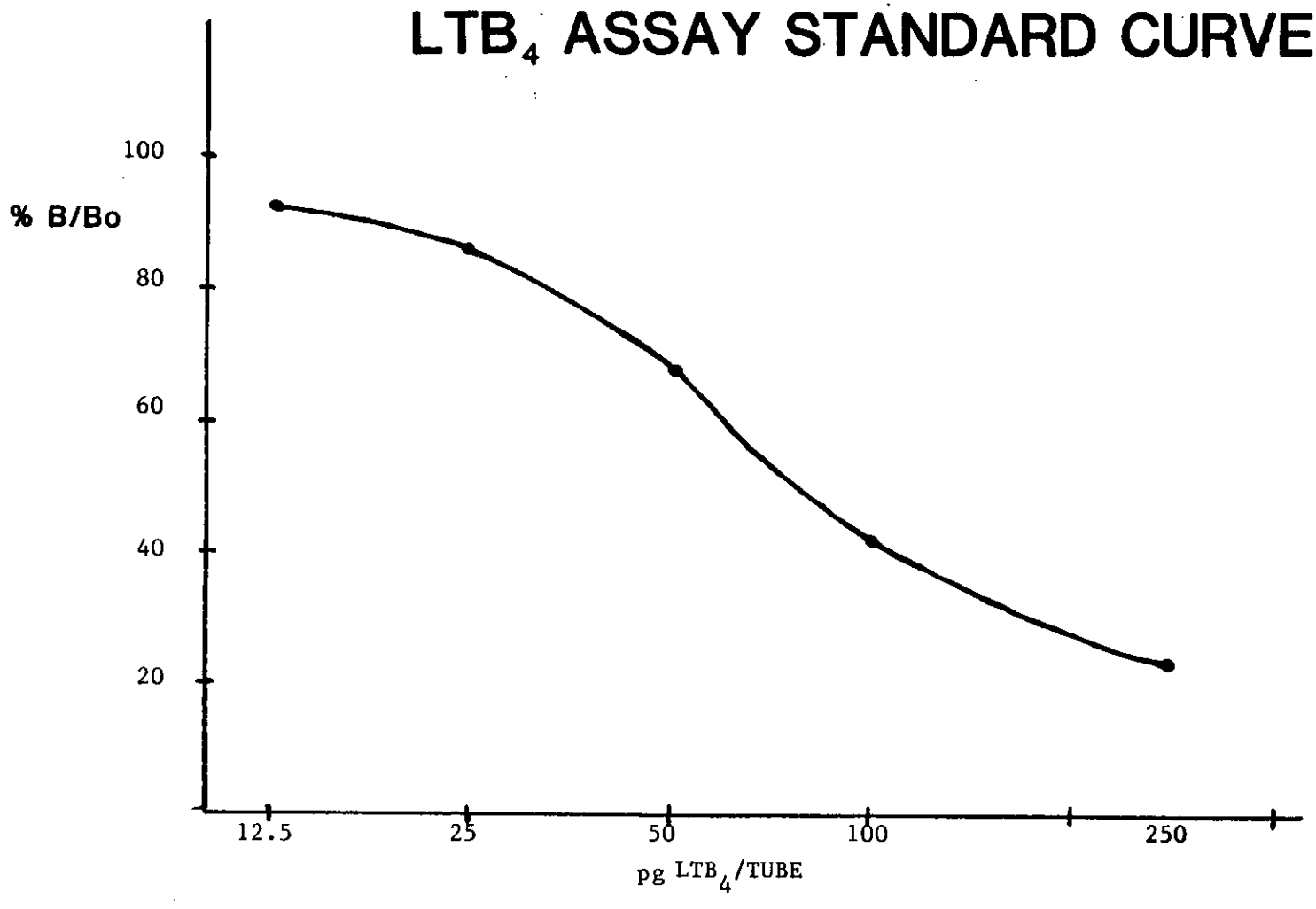

LTB4 ASSAY STANDARD CURVE
% B/Bo
100
80
60
40
20
12.5
25
50
100
250
pg LTB4/TUBE

Figure 7 Standard curve of LTC_4 RIA. Values of LTC_4 for unknown samples were interpolated from a standard curve determined by assaying known quantities of LTC_4 and plotting concentration (picograms LTC_4/ tube) versus mormalized percent bound (% B/Bo). Incubation time: 20 hrs.

LTC_4 ASSAY STANDARD CURVE

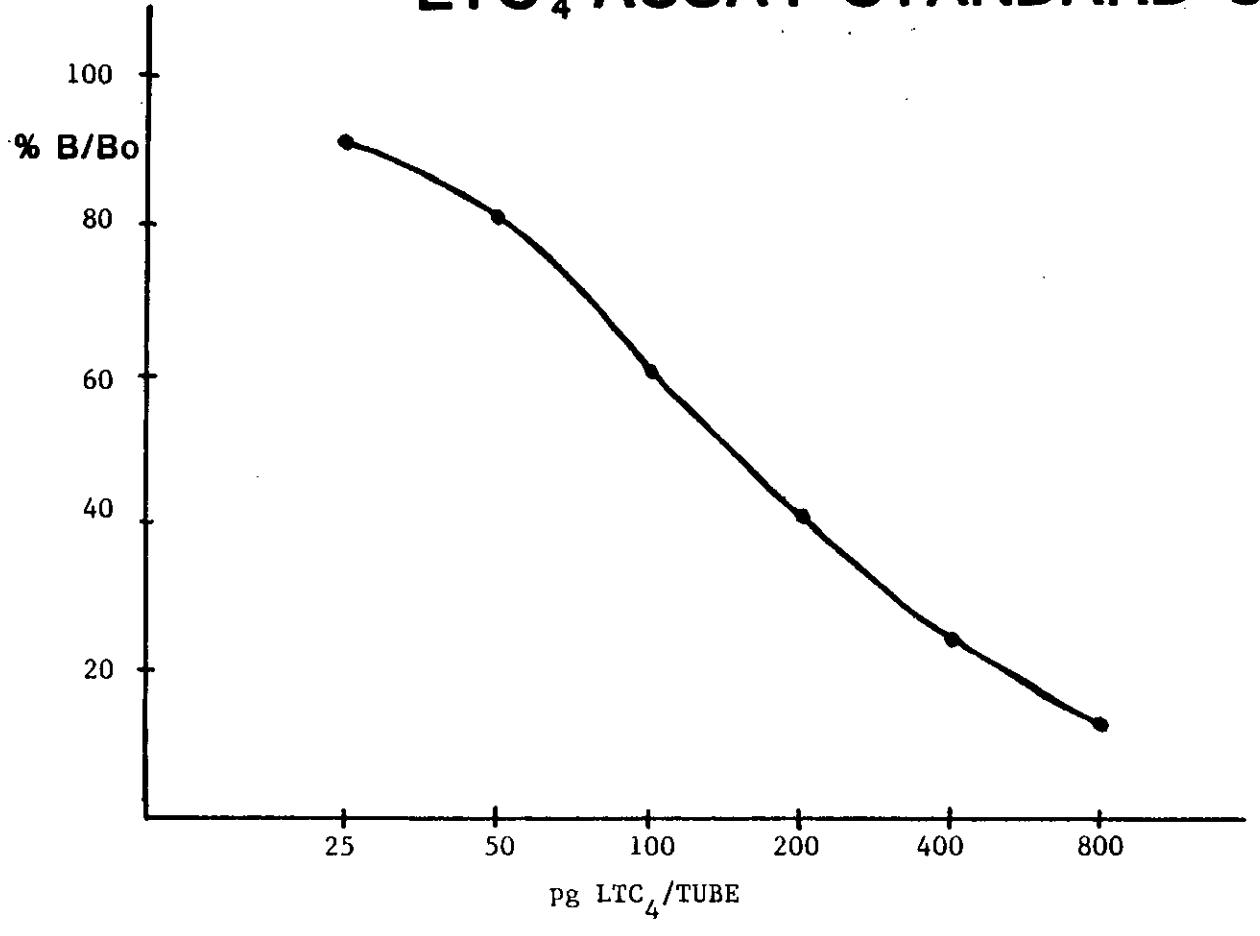


Figure 8 Standard curve of PGE_2 RIA. Values of PGE_2 for unknown samples were interpolated from a standard curve determined by assaying known quantities of PGE_2 and plotting concentration (picogram PGE_2/tube) versus mormalized percent bound (% B/Bo).[2]

Incubation time: 22 hrs.

PGE_2 ASSAY STANDARD CURVE

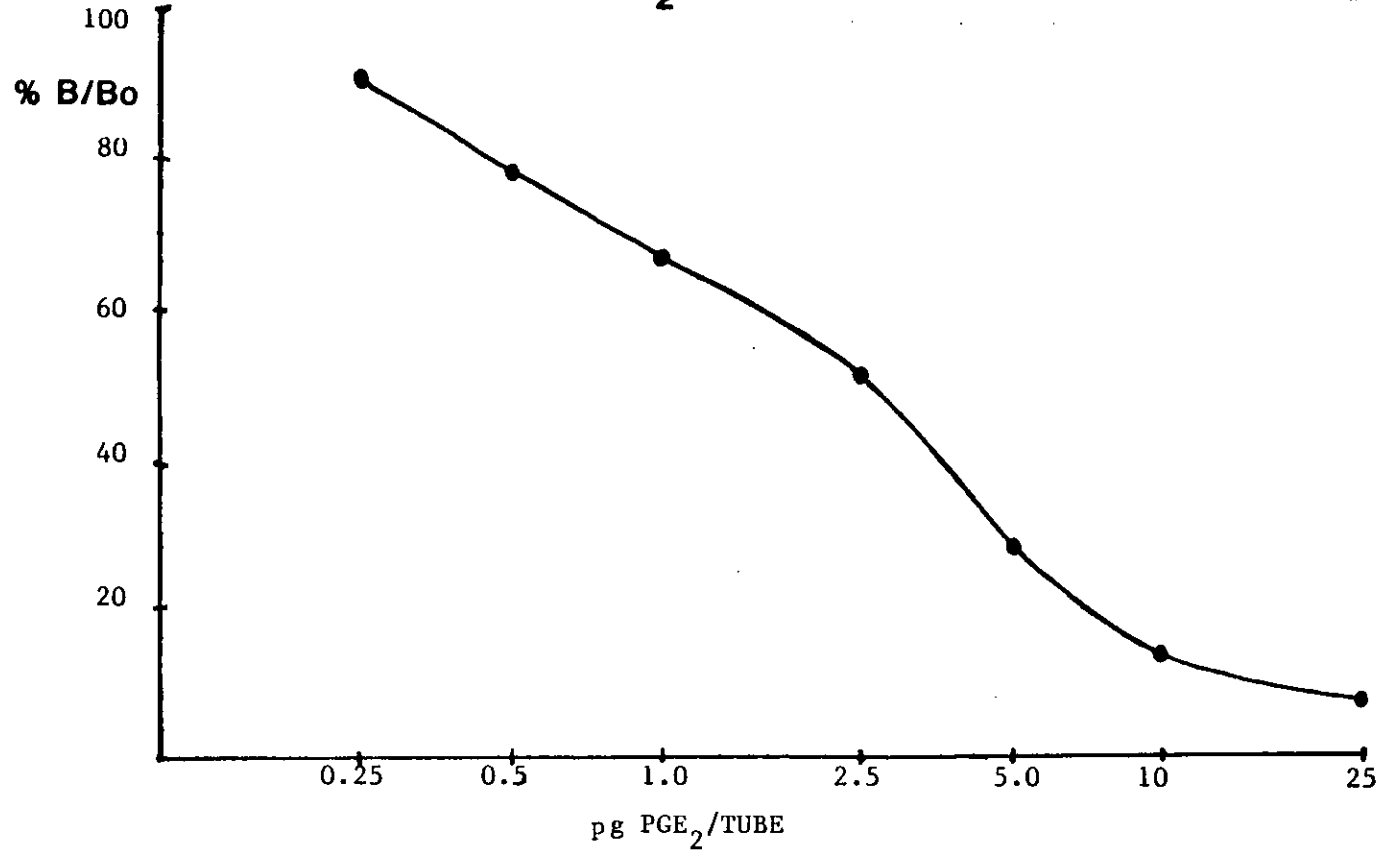
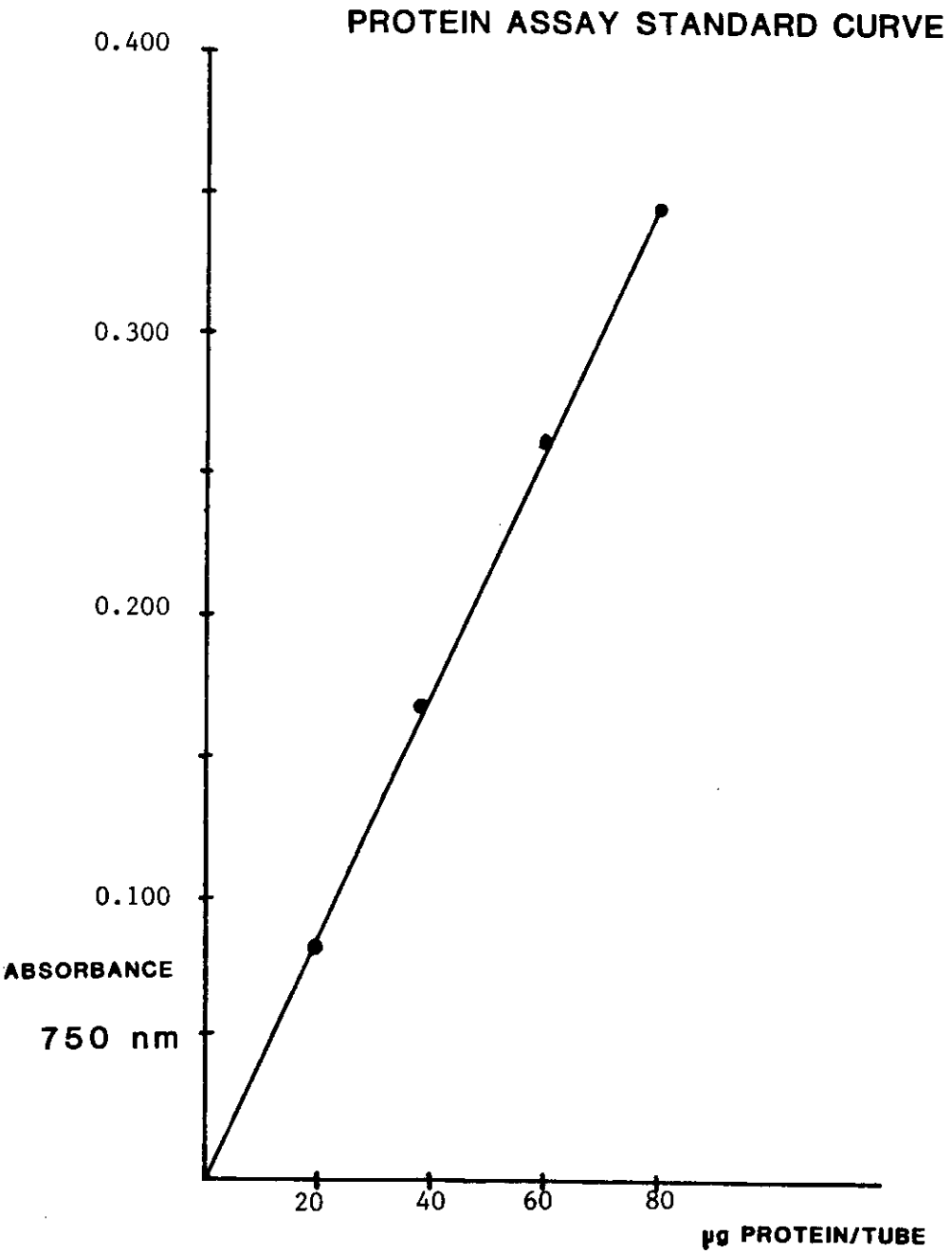


Figure 9 The bovine serum albumin standard curve for Folin non-collagenous protein determination was constructed by determining the absorbance at 750 nm using dilutions of 1.0 mg/ml standard BSA solution versus the absobance.


PROTEIN ASSAY STANDARD CURVE
0.400
0.300
0.200
0.100
ABSORBANCE
750 nm
20
40
60
80
µg PROTEIN/TUBE

prepared (mix 2.0 ml 2.68% NaK tartrate $4H_2O$ + 2.0 ml 1% $CuSO_4 \cdot 5H_2O$ + 100 ml 2% Na_2CO_3 in 0.1 N NaOH). The final reagent added was 0.5 ml of a diluted phenol reagent (phenol Reagent Solution 2N Folin Ciocalteau, Fisher) (1:1 with double distilled water). As the reagent was added it was instantly mixed on a Vortex mixer. After a minimum 1 hour color development time each sample was read for absorbance at 750 nm using a Beckman Spectrophotometer (Model 25, Beckman Instruments, Inc., Irvine, CA). The protein content of the samples was calculated from a blank-corrected standard curve (Fig. 9)prepared from dilutions of a 1.0 mg/ml bovine serum albumin (Crystallized lyophilized, Sigma.

RESULTS

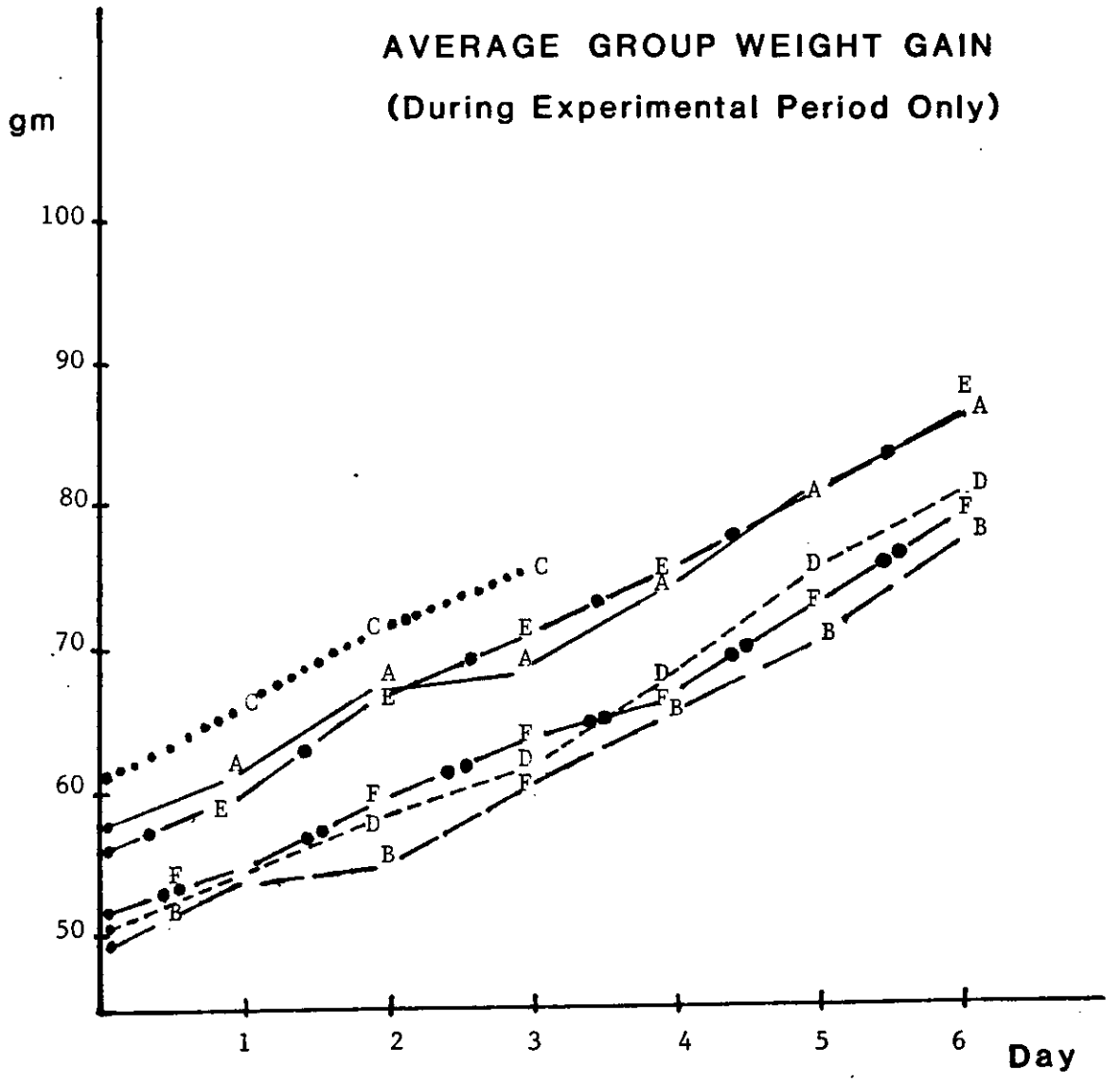
Response of the Animals to the Experimental Procedures

According to the smooth growing record (Fig. 10) and observation on the animals, all rats were adapted to the new environment well after delivery. They also recovered rapidly within one hour after surgical procedures. All animals appeared to be healthy and acted normally after surgery. The wounds were all dried and there was no visible exudates. All the stiches were tight, dried and were well attached to the skin.

Group A showed decreased average growing weight on the third day after surgery. It also happened to group B on the second day after surgery. Actually, this happened on the same day when the animal room was disturbed by the cleaning work to the room. But they were all able to catch up the normal average weight gaining after the annoyance. The average daily group weight gain was 5.0 g/day (Figure 10 & 11). No significant difference in daily weight gain between each groups. The 5.0 g daily weight gain was close to 4.9 g of Radulovich and was less than 5.7 g observed by Collins.

Figure 10 Average daily group weight for the experimental animals during the 6-day experimental period.

The weight of each of the 6 animals in a group was recorded daily and the values were averaged.



A - CONTROL

B - CONTROL + 3 DAYS PIRIPROST

C - 3 DAYS PRESSURE

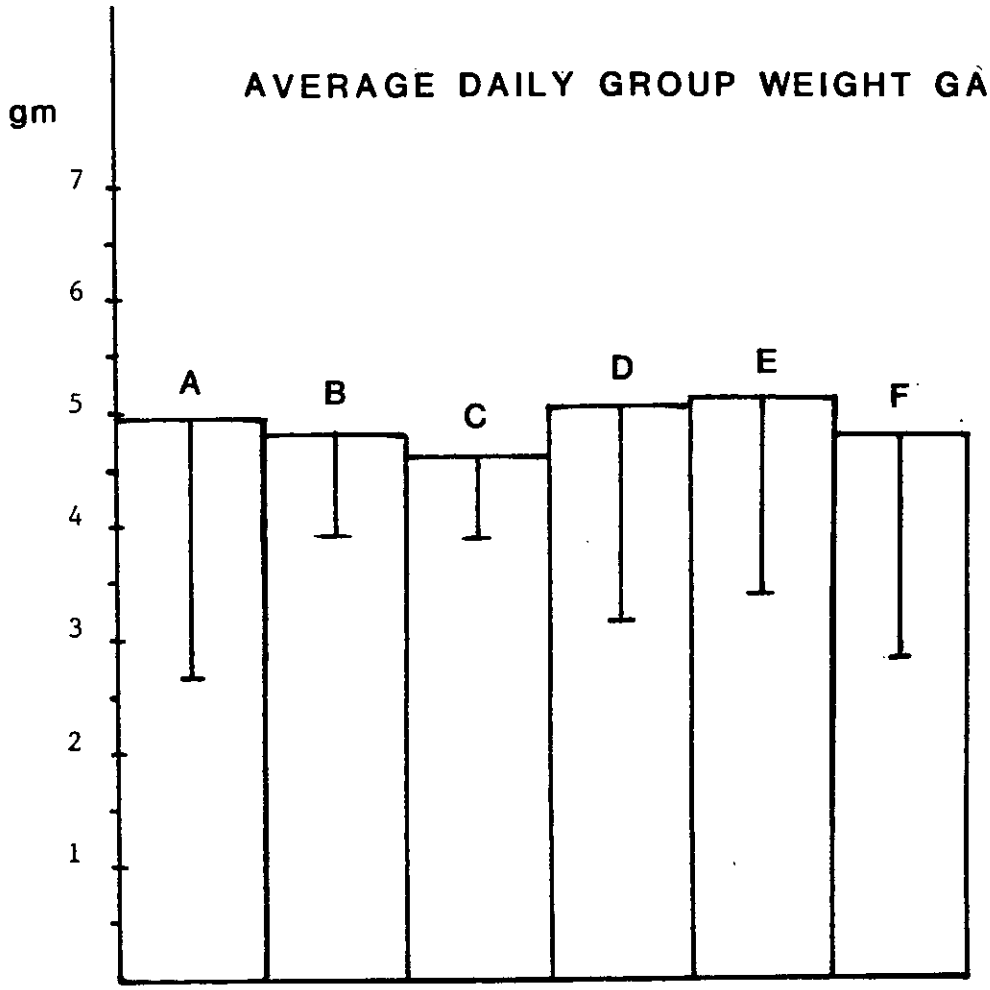
D - 6 DAYS PRESSURE

E - 6 DAYS PRESSURE + 3 DAYS PIRIPROST

F - 6 DAYS PRESSURE + 3 DAYS PIRIPROST & INDOMETHACIN

Figure 11 Average daily group weight gain of 6 animals in each group during the 6 days experiment.

Values were determined by averaging the daily increments of weight gain for each animal in a group. Error bars represent the mean minus the standard deviation.



A - CONTROL

B - CONTROL
+
3 DAYS PIRIPROST

C - 3 DAYS PRESSURE

D - 6 DAYS PRESSURE

E - 6 DAYS PRESSURE
+
3 DAYS PIRIPROST

F - 6 DAYS PRESSURE
+
3 DAYS PIRIPROST
& INDOMETHACIN

Tissue Reaction to Spring Implantation

The tissue response to the spring implantation was by forming loose connective tissue to surround the implanted spring. All the implanted springs were checked to be well seated in position by lifting the head through the main coils of spring. After checking the seating, the spring was carefully removed along with the tangled loose connective tissue. The removed springs were checked and found that the compressive force was reduced to about 10 g when the two legs were expanded 5 mm apart.

Protein Content and Total Bone Weight

All the three eicosanoid contents in the sutural samples were quantitated utilizing radioimmunoassay procedures. In order to compare samples and groups accurately, it is essential to express them on a common basis. Non-collagenous protein content, as determined by the Folin protein assay procedure described previously, can be used to normanize those metabolites data on the basis of active sutural tissue.

In this study, the protein content of the samples were between 4.4% to 11.7% of the total bone weight (see Table II). This variation highlights the inaccuracy if total bone weight is used as comparable basis. Control and those groups treated with piriprost (Groups A, B, E)

TABLE II

TOTAL BONE AND PROTEIN CONTENT OF THE EXPERIMENTAL SAMPLES

Condition	Sample	Animal wt(g) at sacrifice	Total bone wt (mg)	Total protein wt (mg)	mg Protein / mg Bone
Control	A1	80.5	7.4	0.597	0.083
	A4	84.5	5.2	0.488	0.092
	A6	90.0	8.4	0.378	0.044
	A7	80.0	6.8	0.578	0.085
Control + 3 Days Piriprost	B1	80.5	8.4	0.628	0.074
	B2	70.5	7.2	0.446	0.062
	B3	68.0	6.4	0.460	0.073
	B7	92.5	7.3	0.481	0.066
3 Days Pressure	C2	81.5	8.2	0.342	0.045
	C3	79.5	9.2	0.386	0.042
	C4	72.5	8.4	0.413	0.053
	C5	72.0	8.3	0.367	0.049
6 Days Pressure	D1	95.5	9.9	0.596	0.059
	D2	71.0	8.7	0.516	0.060
	D4	78.0	7.3	0.354	0.052
	D5	73.5	8.3	0.405	0.050
6 Days Pressure + 3 Days Piriprost	E3	85.0	12.1	0.960	0.096
	E4	87.5	9.2	0.913	0.117
	E5	88.5	10.4	0.730	0.076
	E6	86.5	7.6	0.574	0.082
6 Days Pressure + 3 Days Piriprost and Indomethacin	F1	78.5	12.7	0.636	0.062
	F2	79.0	9.2	0.645	0.075
	F3	80.0	11.2	0.428	0.047
	F4	78.0	9.9	0.430	0.050

The protein/bone ratio was used to calculate LTB_4, LTC_4 and PGE_2 values in the active bone tissue.

Detail condition see Table I.

TABLE III

TOTAL LTB_4, LTC_4 and PGE_2 LEVELS PER SAMPLE

Condition	Sample	Total LTB_4 (pg)	Group Mean LTB_4 (pg)	Total LTC_4 (pg)	Group Mean LTC_4 (pg)	Total PGE_2 (pg)	Group Mean PGE_2 (pg)
Control	A1	374	294	*	254	444	181
	A4	251		261		56	
	A6	313		355		16	
	A7	239		146		209	
Control + 3 Days Piriprost	B1	659	83.9 (n=3) 315 (n=4)	429	497	75	132
	B2	176		496		175	
	B3	0		892		277	
	B7	73		169		2.8	
3 Days Pressure	C2	275	21.9	137	333	255	297
	C3	156		202		118	
	C4	308		181		354	
	C5	138		486		461	
6 Days Pressure	D1	253	172	0	271	235	201 (n=3) 426.8 (n=4)
	D2	0		380		260	
	D4	302		317		107	
	D5	131		387		1104	
6 Days Pressure + 3 Days Piriprost	E3	218	211	457	170	497	321.6
	E4	177		0		282	
	E5	187		223		305	
	E6	261		0		203	
6 Days Pressure + 3 Days Piriprost and Indomethacin	F1	203	144	276	107	0	29.0
	F2	122		154		56.1	
	F3	197		0		21.3	
	F4	55.4		0		38.6	

*Sample lost during evaporation

TABLE IV

LTB_4, LTC_4 AND PGE_2 LEVELS PER BONE WEIGHT

Condition	Sample	LTB_4 pg/mg bone	Group Mean LTB_4 pg/mg bone	LTC_4 pg/mg bone	Group Mean LTC_4 pg/mg bone	PGE_2 pg/mg bone	Group Mean PGE_2 pg/mg bone
Control	A1	50.5	42.8	*	38.0	60.0	25.9
	A4	48.3		50.2		10.8	
	A6	37.2		42.3		1.9	
	A7	35.2		21.5		30.8	
Control + 3 days Piriprost	B1	82.7	29.3	51.5	70.7	8.9	19.2
	B2	24.4		68.9		24.3	
	B3	0		139.4		43.2	
	B7	10.0		23.1		0.38	
3 days Pressure	C2	33.5	25.9	16.7	20.0	31.1	35.4
	C3	16.9		22.0		12.8	
	C4	36.7		21.5		42.1	
	C5	16.6		58.5		55.5	
6 Days Pressure	D1	25.5	20.7	0	32.0	23.7	22.8 (n=3)
	D2	0		38.4		29.9	
	D4	41.4		43.4		14.7	50.3 (n=3)
	D5	15.8		46.6		133	
6 Days Pressure + 3 Days Piriprost	E3	18.0	22.5	37.8	14.8	41.1	31.9
	E4	19.2		0		30.6	
	E5	18.0		21.4		29.3	
	E6	34.7		0		26.7	
6 Days Pressure + 3 Days Piriprost and Indomethacin	F1	16.0	13.1	21.7	9.6	0	3.0
	F2	13.3		16.7		6.1	
	F3	17.6		0		1.9	
	F4	5.6		0		3.9	

* Sample lost during evaporation

These values are presented for comparison with Table V.

TABLE V

LTB_4, LTC_4 and PGE_2 PER PROTEIN WEIGHT

Condition	Sample	LTB_4 pg/mg protein	Group Mean LTB_4 pg/mg protein	LTC_4 pg/mg protein	Group Mean LTC_4 pg/mg protein	PGE_2 pg/mg protein	Group Mean PGE_2 pg/mg protein
Control	A1	608		**		723	
	A4	525	598	546	587	117	312
	A6	846		961		43	
	A7	414		253		362	
Control	B1	*1118	182	691		120	
+ 3 Days	B2	394	(n=3)	1111	1015	392	278
Piriprost	B3	0	*416	1910		592	
	B7	152	(n=4)	350		6	
3 Days Pressure	C2	745		371		691	
	C3	402	545	523	623	305	731
	C4	693		406		794	
	C5	339		1194		1133	
6 Days Pressure	D1	432	515	0	802	402	394
	D2	* 0	(n=3)	640	(n=3)	498	(n=3)
	D4	796	*386	835	602	283	*961
	D5	316	(n=4)	932	(n=4)	2260	(n=4)
6 Days Pressure	E3	188		394		428	
+ 3 Days	E4	164	253	0	169	262	350
Piriprost	E5	237		282		386	
	E6	423		0		326	
6 Days Pressure	F1	254		344		0	
+ 3 Days	F2	177	230	355	275	81	50
Piriprost and	F3	377		0		40	
Indomethacin	F4	112		0		78	

*Deleted for statistics because of abnormal values
**Sample lost during evaporation

Only data expressed in terms of protein weight were used for statistical analyses.

All values are adjusted to the following Extraction Recovery Rate: LTB_4 = 62.0%
LTC_4 = 80.0%
PGE_2 = 88.0%

TABLE VI

SUMMARY OF LTB_4, LTC_4 AND PGE_2 ANALYSIS

Condition	LTB_4 Mean pg/mg Protein ± SD	LTB_4 % of Control	LTC_4 Mean pg/mg Protein ± SD	LTC_4 % of Control	PGE_2 Mean pg/mg Protein ± SD	PGE_2 % of Control
Control	598 ± 183	100%	578 ± 356	100%	312 ± 306	100%
Control + 3 Days Piriprost	*142 ± 198 416 ± 495	30%	1015 ± 673	173%	278 ± 265	89%
3 Days Pressure	545 ± 204	91%	623 ± 386	106%	731 ± 341	234%
6 Days Pressure	*515 ± 250 386 ± 329	86%	*802 ± 149 602 ± 419	137 %	*394 ± 108 960 ± 1136	126%
6 Days pres. + 3 Days Piriprost	253 ± 117	42%	169 ± 200	29%	350 ± 72	112%
6 Days Pres. + 3 Days Piriprost & Indomethacin	230 ± 114	38%	175 ± 202	30%	50 ± 38	16%

This Table summerizes the average LTB_4, LTC_4 and PGE_2 values for each condition plus % of Control

*n=3 to be used in % of Control, Bar graphs and statistics, the fourth abnormal value was deleted.

TABLE VII

ANALYTICAL STATISTICS STUDENT T-TEST

LTB_4 (pg/mg Protein)

MEASUREMENT	n_1	n_2	x_1	x_2	s_1	s_2	t	α
A to B	4	3	598	182	183	198	3.086	0.05
A to D	4	3	598	515	183	250	0.536	NS
B to E	3	4	182	253	198	117	0.617	NS
C to D	4	3	545	515	204	250	0.186	NS
D to E	3	4	515	253	250	117	1.899	NS
D to F	3	4	515	230	250	114	2.080	NS
E to F	4	4	253	230	117	114	0.281	NS

A - Control

B - Control + 3 Days Piriprost

C - 3 Days Pressure

D - 6 Days Pressure

E - 6 Days Pressure + 3 Days Piriprost

F - 6 Days Pressure + 3 Days Piriprost and Indomethacin

α = Level of significance

NS = Not Significant

TABLE VIII

ANALYTICAL STATISTICS STUDENT T-TEST

LTC_4 (pg/mg Protein)

MEASUREMENT	n_1	n_2	x_1	x_2	s_1	s_2	t	α
A to B	4	4	587	1015	356	673	1.148	NS
A to D	4	3	587	802	356	149	1.161	NS
B to E	4	4	1015	169	673	200	2.410	0.05
C to D	4	3	623	802	386	149	0.865	NS
D to E	3	4	802	169	149	200	5.076	0.01
D to F	3	4	802	175	149	202	5.016	0.01
E to F	4	4	169	175	200	202	0.042	NS

A - Control

B - Control + 3 Days Piriprost

C - 3 Days Pressure

D - 6 Days Pressure

E - 6 Days Pressure + 3 Days Piriprost

F - 6 Days Pressure + 3 Days Piriprost and Indomethacin

α = Level of significance

NS = Not Significant

TABLE IX

ANALYTICAL STATISTICS STUDENT T-TEST

PGE_2 (pg/mg Protein)

MEASUREMENT	n_1	n_2	x_1	x_2	s_1	s_2	t	α
A to B	4	4	312	278	306	265	0.168	NS
A to D	4	3	312	394	306	108	0.506	NS
B to E	4	4	278	350	265	72	0.524	NS
C to D	4	3	731	394	341	108	1.885	NS
D to E	3	4	394	350	108	72	0.678	NS
D to F	3	4	394	50	108	38	6.014	0.01
E to F	4	4	350	50	72	38	7.371	0.01

A - Control

B - Control + 3 Days Piriprost

C - 3 Days Pressure

D - 6 Days Pressure

E - 6 Days Pressure + 3 Days Piriprost

F - 6 Days Pressure + 3 Days Piriprost and Indomethacin

α = Level of Significance

NS = Not Significant

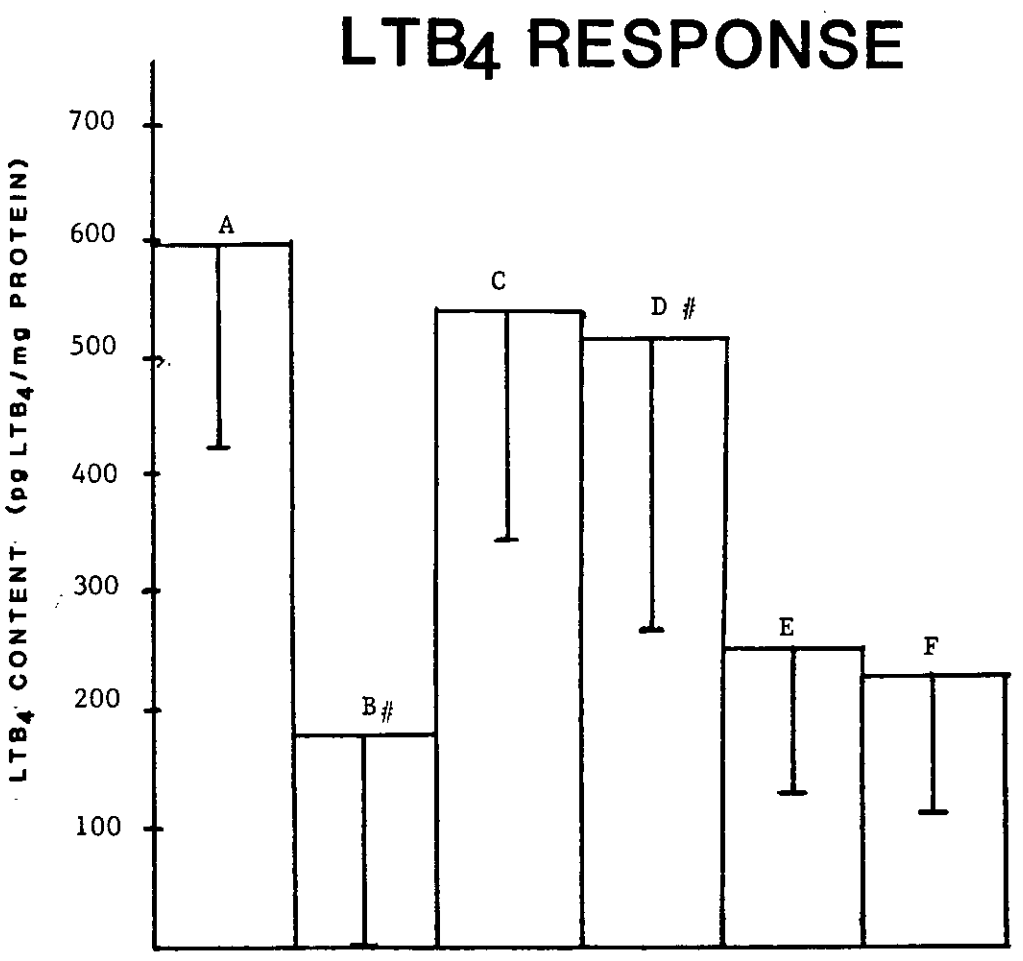
had higher protein content per bone weight. This may relate to the higher cellularity found in group B and E.

Analysis of LTB_4, LTC4, and PGE_2 Response

Table IV and V represent the LTB_4, LTC_4 and PGE_2 data for each sample expressed in per mg of bone and per mg of protein, respectively. The mean values, with standard deviations, of the metabolite responses are summerized in Table VI. These values are depicted in bar graphs for each of the metabolite in Figure 12, 13 and 14 and are summerized in Figure 15. For statistical analysis the student t-test was utilized. the 0.05 confidence level was used to test for significane (Table VII, VIII and IX). LTB_4 data from animal B1, D2 were deleted for statistical analysis due to either unreasonable too high or too low LTB_4 level. LTC_4 data from D5 were also deleted due to the same reason. Iatrogenic handling of the samples during removal and during RIA procedures may have caused those large variations which are not representative of the experimental conditions.

LTB_4 responses (Figure 12) from 3 days and 6 days pressure groups were 91% and 86% respectively compared to control. These slight difference were not statistically significant. The groups treated with piriprost showed significant decrease in the content of LTB_4 when compared

Figure 12 Average group response of LTB_4 to experimental conditions. Error bars represent the mean minus the standard deviation. # N=3. Data from Table VI



A - CONTROL

B - CONTROL
+
3 DAYS PIRIPROST

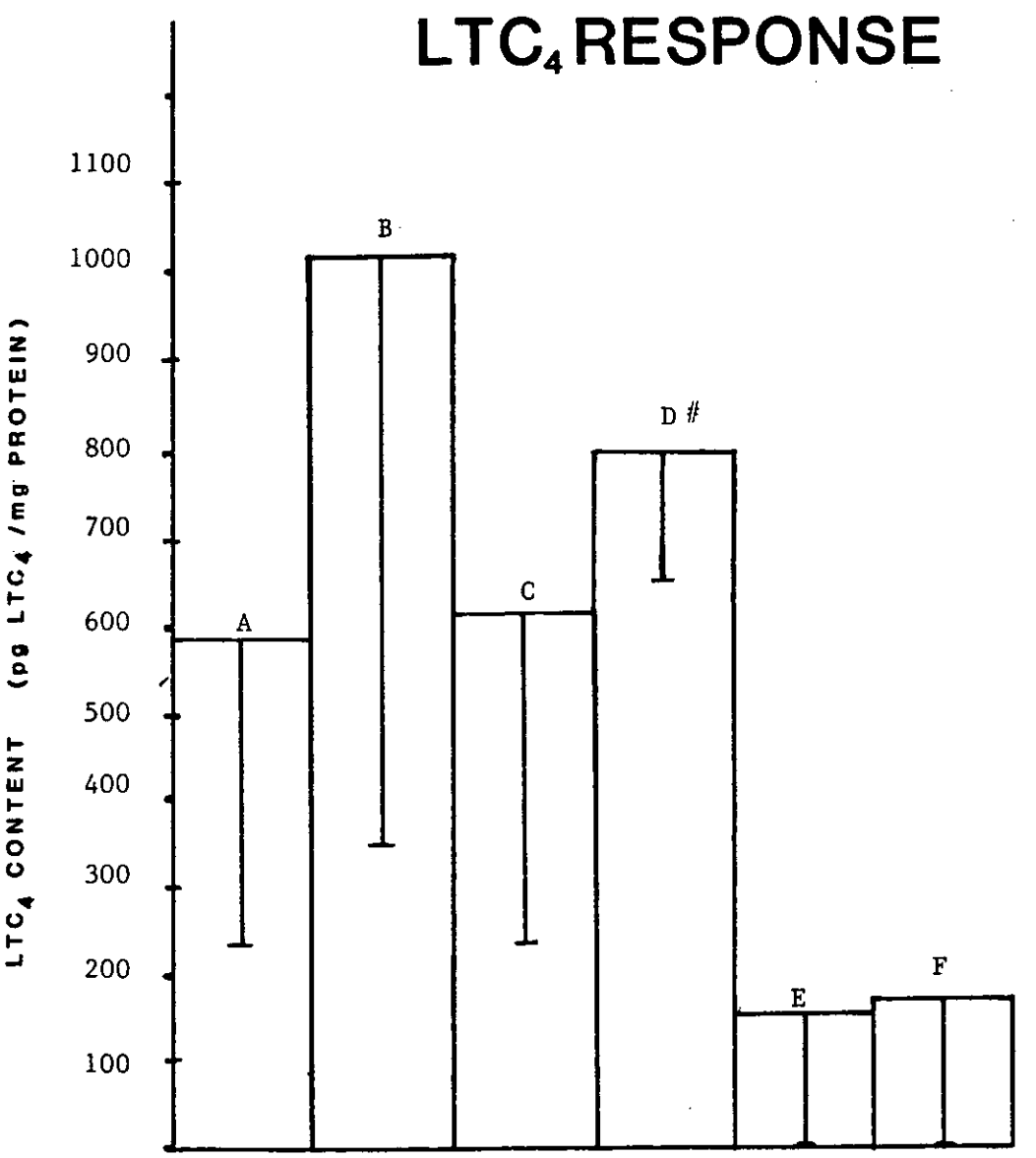
C - 3 DAYS PRESSURE

D - 6 DAYS PRESSURE

E - 6 DAYS PRESSURE
+
3 DAYS PIRİPROST

F - 6 DAYS PRESSURE
+
3 DAYS PIRIPROST
& INDOMETHACIN

Figure 13 Average group response of LTC_4 to experimental conditions. Error bars represent the mean minus the standard deviation. # N=3. Data from Table VI



A - CONTROL

B - CONTROL + 3 DAYS PIRIPROST

C - 3 DAYS PRESSURE

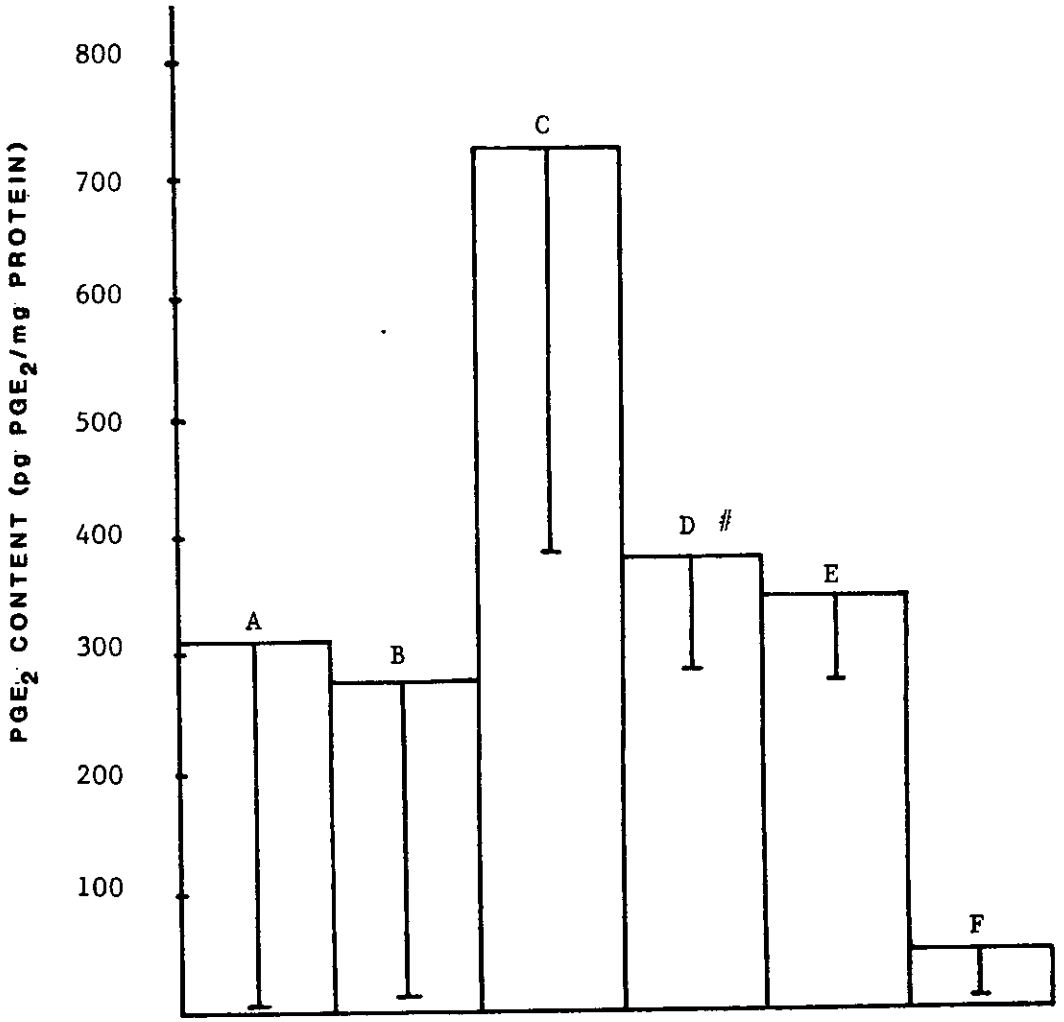
D - 6 DAYS PRESSURE

E - 6 DAYS PRESSURE + 3 DAYS PIRIPROST

F - 6 DAYS PRESSURE + 3 DAYS PIRIPROST & INDOMETHACIN

Figure 14 Average group response of PGE_2 to experimental conditions. Error bars represent the mean minus the standard deviation. # N=3. Data from Table VI

PGE_2 RESPONSE



A - CONTROL

B - CONTROL + 3 DAYS PIRIPROST

C - 3 DAYS PRESSURE

D - 6 DAYS PRESSURE

E - 6 DAYS PRESSURE + 3 DAYS PIRIPROST

F - 6 DAYS PRESSURE + 3 DAYS PIRIPROST & INDOMETHACIN

Figure 15 A composite of LTB_4, LTC_4 and PGE_2 responses.

Values determined as a function of % of controls for LTB_4, LTC_4 and PGE_2. Data from Table VI

A = Control osteotomy 6 days

B = Control + Piriprost

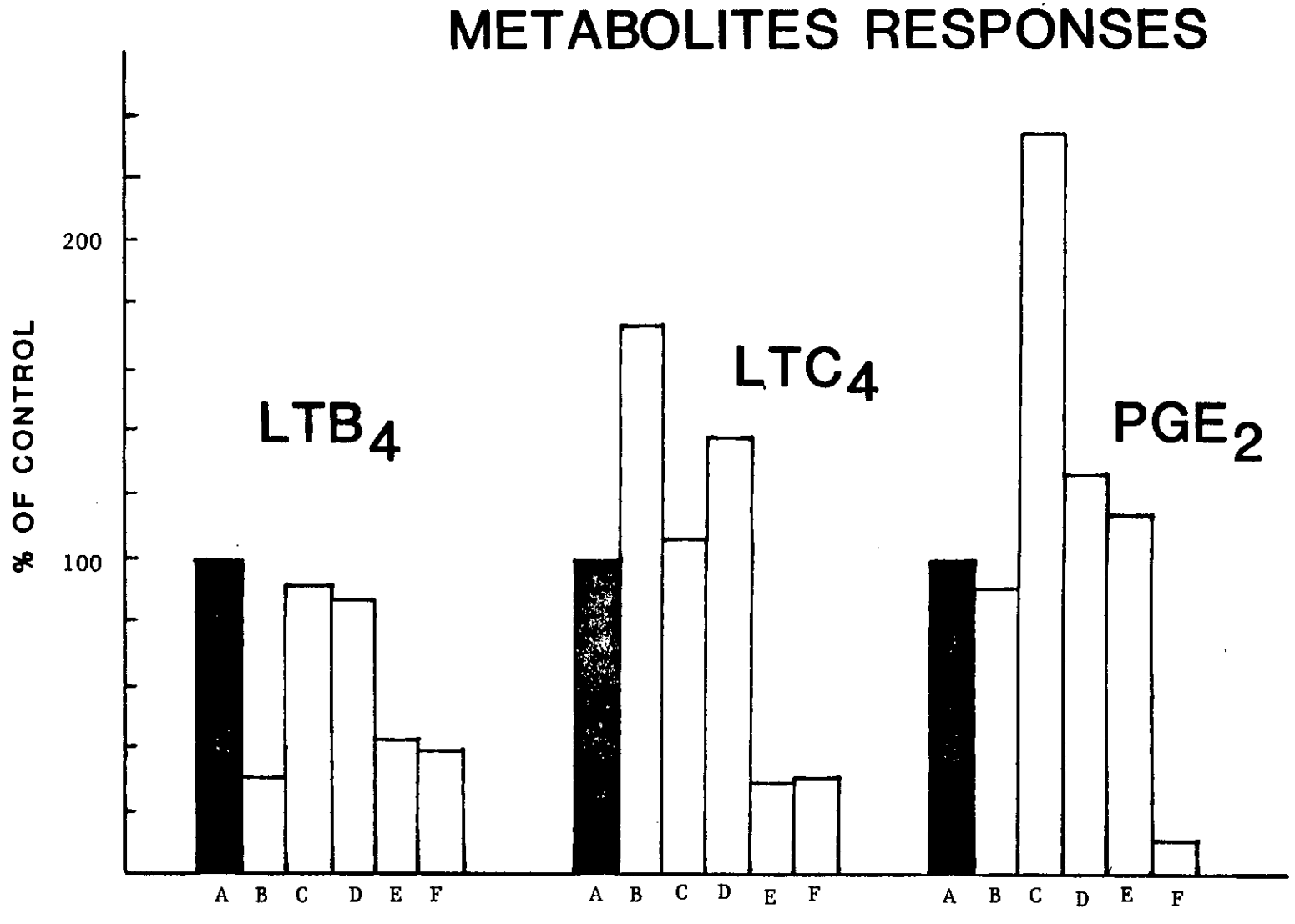
C = 3 days pressure

D = 6 days pressure

E = D + 3 days piriprost

F = E + 3 days indomethacin

Detail design see Table I


METABOLITES RESPONSES
% OF CONTROL
200
100
LTB4
LTC4
PGE2
A B C D E F
A B C D E F
A B C D E F

to control. The applied 20 g pressure showed no effect to change the LTB_4 contents either among the piriprost treated (Group B, E, and F) or among nonpiriprost treated groups (Groups A, C and D). Although Group E and F showed slight higher contents than Group B did, there was no significant difference between these two different treatments (with and without pressure).

LTC_4 response (Figure 13) for Group B elevated 73% over control. Group D was 37% over control. But Group B, C, and D all showed no statistical signifcance to control. Group E and F which receive pressure and piriprost had significant lower level of LTC_4 compared to control, to pressure free control (Group B) and to drug free groups (Group C and D). There was no significant difference between the two experimental groups (E and F) whether treated with indomethacin or not.

The PGE_2 responses were shown in Figure 14. Three days pressure resulted in two fold elevation of PGE_2 response when compared to 6 days control and to 6 days pressure. The piriprost treated groups (B and E), no matter treated with pressure or not, showed no significant difference when compared with control. Group F, when compared to control and Group E, had significant low level of PGE_2 because of treatment of indomethacin.

Histological Results

Histological samples were embedded in maraglass (epoxy plastic embedding) and were sectioned in ultra thin thickness (1 μm). They were then stained with toludin blue O (TBO) and alizarin red S (ARS). TBO stains cell membrane, nuclei and alkaline particles and shows very good details. ARS gives calcified tissue light to dark reddish brown color according to the degree of calcification. The combination of the two stains has proved to be superior to the H&E stain in the hard tissue because TBO and ARS staining methods do not require decalcification and thus preserved more detail strutures.

Histological evaluation was based upon (1) overall sutural appearance, (2) evidence of bone formation and/or bone resorption, and (3) cellular appearance including inflammatory response.

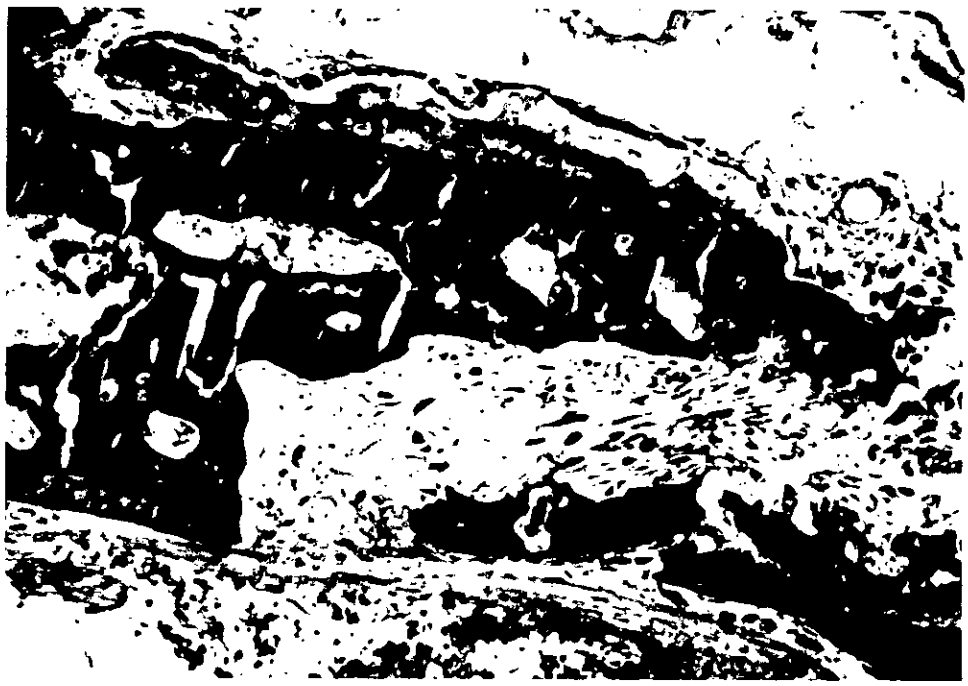
The overall picture of control is seen in plate V , the sutural width in the control was used as a comparative baseline. The bony plate in both sides of the suture had overlapping relation but the tissue showed no tension nor compression. The ectocranial periosteal surface had evidence of bone apposition (osteoid tissue laid) while about half of the sutural area showed slight to moderate degree of bone resorption with a few osteoclasts. The medullary spaces were lined up with osteoblast-like cells and showed no sign of resorption

Plate V 6 days osteotomy Control group (Group A)

Slight to moderate resorptive system has been developed in the suture. The ectocranial bony surface and periosteum showed fast deposition of osteoid tissue. The vessel in the right upper corner was midsagittal artery which serves good landmark of sutural position. The bony plate in the right lower corner was fractured during sample processing which causes false twisting appearance of the sutural fiber bundles. (160 X, Toluidin blue O & Alizarin red S stain)

Plate VI Control + Piriprost for the last 3 days (Group B)

The picture showed same degree of bone apposition (osteoid laid on the ectocranial surface, upper surface) and bone resorption as seen in Control. The cellularity was high and the cells were dark stained and ovoid compared to Control. (160 X, Toluidin blue O and Alizarin red S stain)



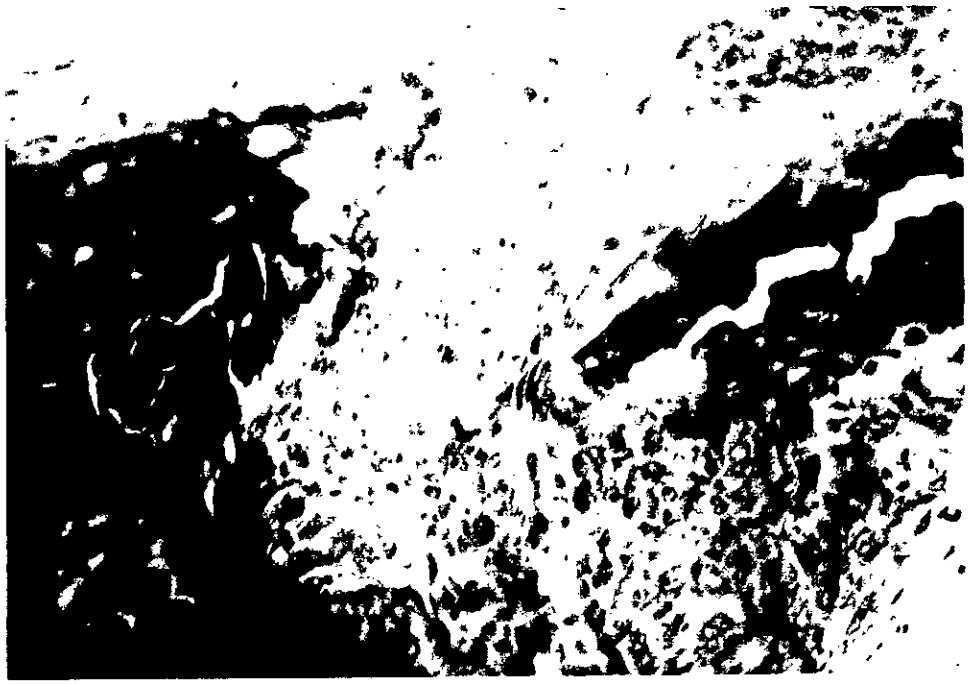


Plate VII Pressure for 3 days only (Group C)

The suture with total of 3 days pressure showed no evidence of resorption. The fiber bundles in the sutural area underwent hyalinization and started to necrosis. Their nuclei were pycnotic. (160 X, Toluidin blue O & Alizarin red S stain)

Plate VIII Pressure for 6 days (Group D)

This group was observed with moderate degree of resorption in the suture and in the medullary spaces. The applied pressure made a narrowed sutural area where contained compressed fiber bundles with some degree of hyalinization. (160 X, Toluidin blue O & Alizarin red S stain)

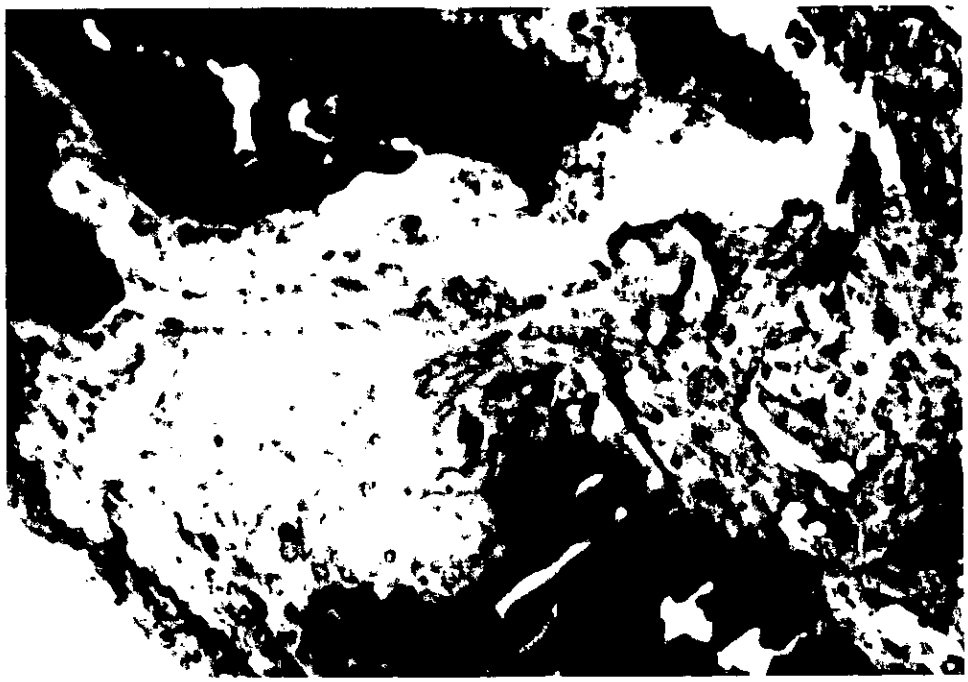




Plate IX 6 days Pressure + Piriprost for the last 3 days (Group E)
The unsmooth surfaces of the bone implies some degree of resorptive activity before. Those surfaces were not occupied by osteoclasts at the time of sacrifice. The picture was like that was seen in Control + Piriprost group B (Plate VI) in terms of high cellularity, dark stained and ovoid matrix cells. (160 X, Toluidin blue O & Alizarin red S stain)

Plate X 400 X Magnification of Plate IX

Note the darker stained ovoid cells tended to be lined along the bony surface.



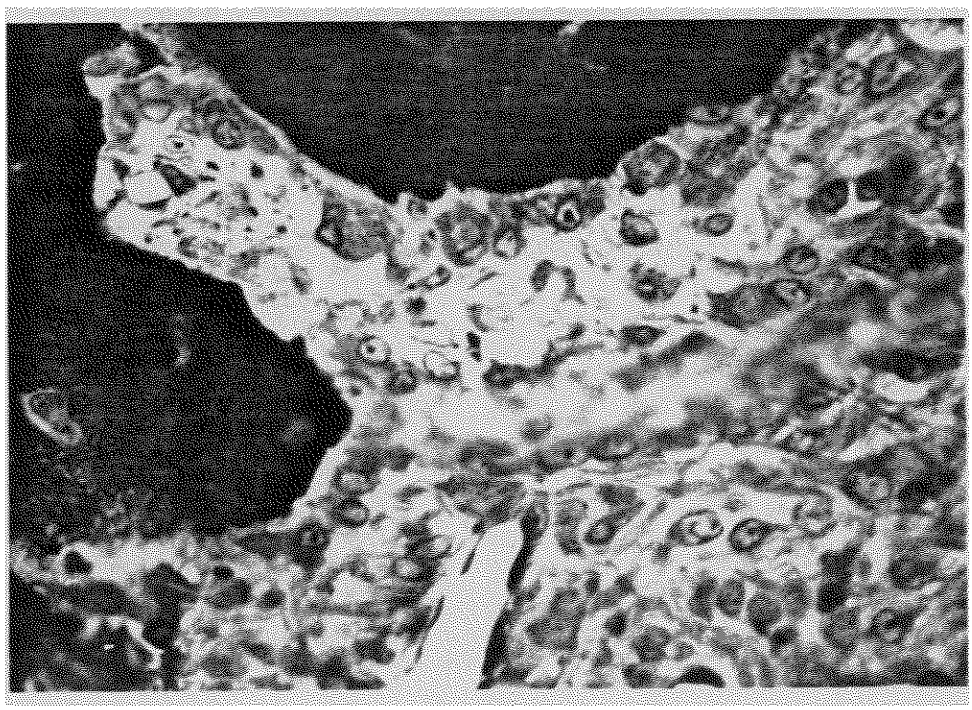


Plate XI 400 X Magnification of Invagination of Bone Surface in Sutural Area. (same sample but different depth of sectioning, Group E)

Note that the darker stained ovoid cells were well lined along the ~~medullary~~ Sutural bone surface.

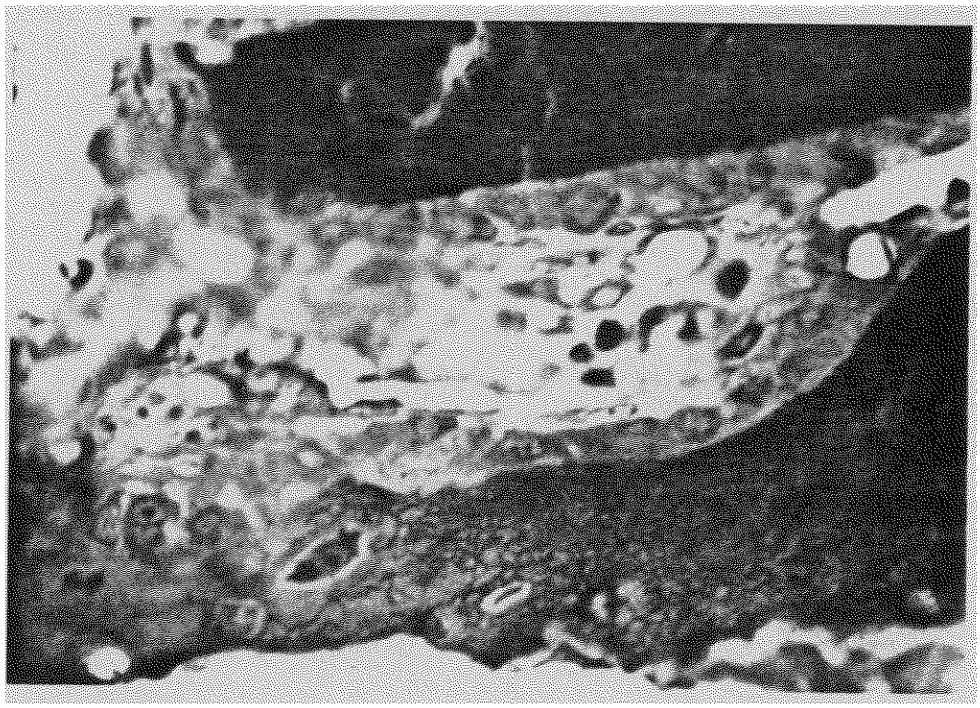
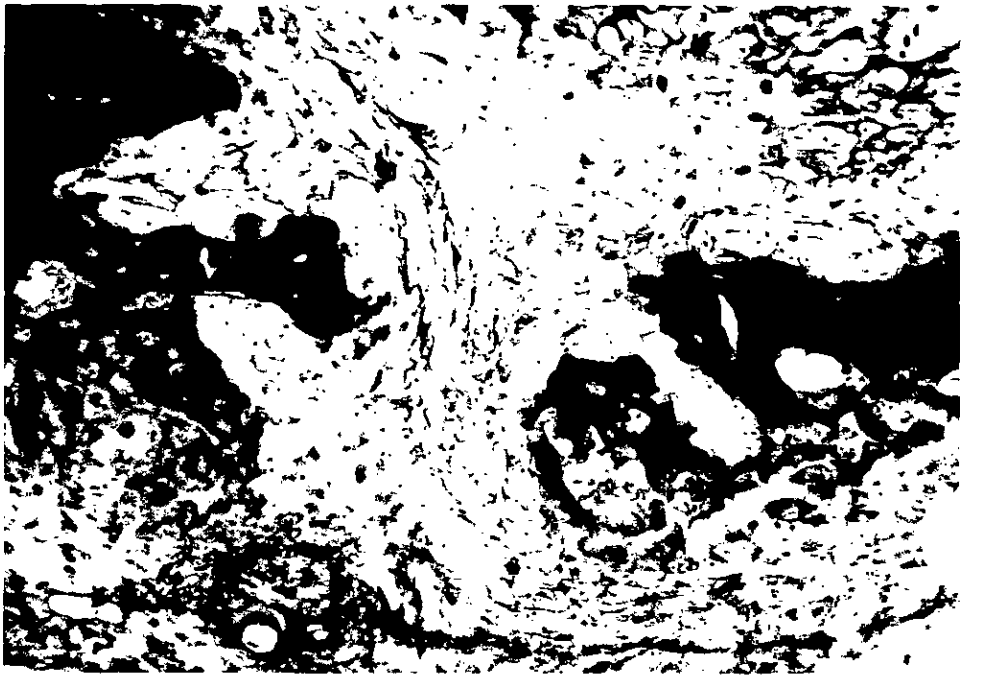


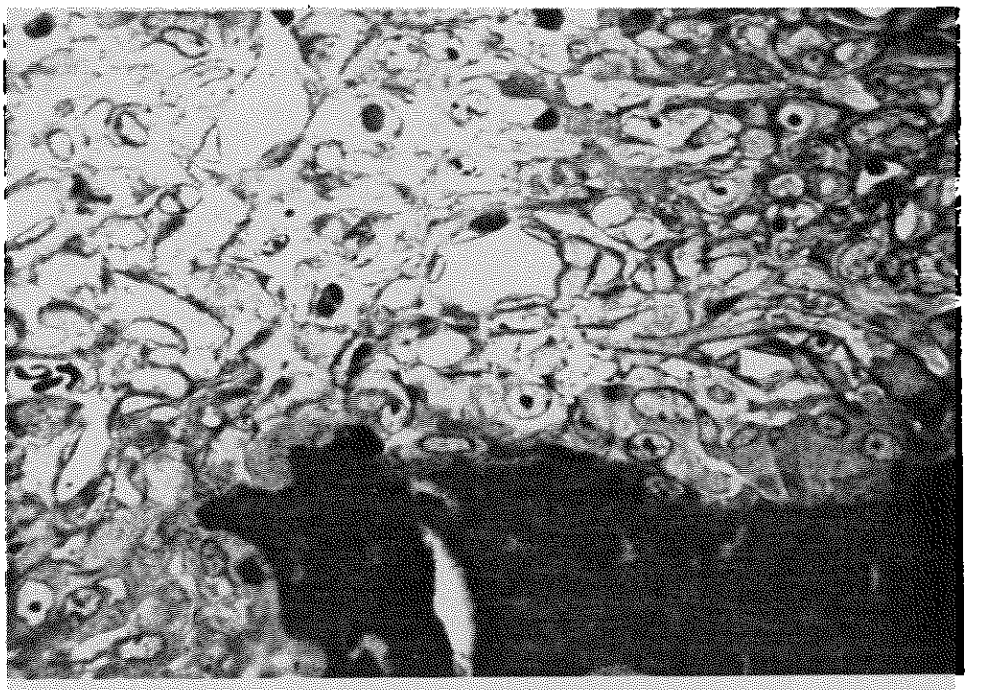
Plate XII 6 days Pressure + Indomethacin and Piriprost for the last 3 days (Group F)

The histology showed active bone remodeling including resorption and deposition in the areas close to the suture. The cells in the suture looked abnormal. The fibroblasts became swollen and showed ghost-like appearance with light stained cytoplasm and nuclei. In addition, the sutural matrix had many vascular spaces. Some of the endothelial cells of the vessels were swollen and were bulging up to the vascular spaces. (160 X)

Plate XIII 400 X Magnification of Plate XI

Note the bulging endothelial cells, and light stained cells. (400 X , Toluidin blue O & Alizarin red S stain)





(ref. Elaine Klingler and Delhi Guillaumin 1985).

Group B (plate VI), which was treated with osteotomy and the last 3 days piriprost injection, had the same degree of resorption as control. The cells lining the sutural area were more rounding and dark stained. The cellular activity was higher than control.

Group C (plate VII), which was treated with total of 3 days pressure, showed no evidence of resorption. The fiber bundles in the sutural area underwent hyalinization and started to necrosis. Their nuclei were pycnotic due to compressive effect. Osteoid substance was deposited on the ectocranial surface of the bone as found in control.

Group D (plate VIII), were treated with total of 6 days pressure and were observed with moderate degree of resorptive appearance in the suture and in the medullary space. The applied pressure made a narrowed sutural area where contained compressed fiber bundles with some degree of hyalinization. The cellularity was the same as control.

Group E (plate IX, X and XI), which were treated with 6 days pressure and last 3 days piriprost injections, showed high cellularity in their sutural area. There were many ovoid basophilic cells lining along the unsmooth sutural bone surface and also along the medullary spaces. The unsmooth surfaces of the bone

might imply some degree of resorptive activity before, yet those surfaces were not occupied by any osteoclast-like cells at the time of sacrifice.

Group F (plate XII and XIII) that were treated with 3 days piriprost and indomethacin and 6 days pressure resulted in narrowed sutures. The histology showed busy bone remodeling including resorption and deposition in the areas close to the suture. The cells in the suture looked abnormal. The fibroblasts became swollen and showed ghost-cell like appearance with light stained cytoplasm and nuclei. In addition, the sutural matrix had many vascular spaces. Some of the endothelial cells of the vessels were swollen and were bulging up to the vascular spaces.

DISCUSSION

The study will be discussed in the following sections: (A) purpose and design of the experiment, (B) materials and methods, (C) results and comparison to Benson (1985) and Collins (1986), (D) clinical significance.

A. Purpose and design of the experiment

The purpose of the experiment was to evaluate the cellular behavior and metabolite contents in the resorption induced sutural system versus Collins' (1986) original formative system under the influence of piriprost and compressive force to the midsagittal suture of rats *in vivo*. The experimental length 6 days was decided because it is easier to divide into two periods, first 3 days resorptive inducing period and last 3 days drug experimental period. The design used by Collins was primarily to evaluate the response of LTB_4, LTC_4 and PGE_2 through 5 days experiment by tension and pressure. Fortunately and interestingly, he found significant cuboidal basophilic cells in the sutural surface and in the medullary spaces in the pressure and piriprost treated groups. The use of two-sectioned experimental periods was to induce resorptive system in the suture prior to administering piriprost and to evaluate the

ability of piriprost to initiate the formative system. Three days piriprost treatment may have just started to induce the resorptive system to depository system. The different timing to evaluate the metabolites and histological behavior were set up in group A, B, C and D (see detail in Table I).

The study used only compressive experiment was again because of the interesting in Collins' finding which was found most strikingly in pressure group. With compressive experiment, seating compressive spring could be more difficult than seating tensive spring. This is because compressive spring tends to twist the suture structure or to overlap the calvarial plates of the suture and thus cause some tension effect on the sutural system. Fortunately, care was taken to avoid this from happening and the results were found only one (E sample) out of eight compresive histological specimens has twisting phenomenon. This specimen was omitted in the description of the result.

It should also be noted that none of tension nor pressure groups will show a pure resorptive or formative system if the sutural system is not a simple butt joint suture.

It is also very interesting to observe the effect, or toxic effect, of combined use of indomethacin and piriprost to inhibit cyclooxygenase acid 5-lipoxygenase

pathways simultaneously on the sutural tissue. Blockage of these two pathways in arachidonic and cascade (Figure 2) may have two folds of meaning: (a) elevating the metabolic contents in 15- and 12- lipoxygenase pathways and (b) blockage of important inflammatory mediators (Willoughby 1972; Samuelsson 1983), LTs and PGs. The effect of (a) and (b) has not been reported in any experiment . The possible predicted effect was reduction in both resorptive and depository bone remodeling activities (see result discussion).

B. Materials and methods

The animals used had average group weight range between 40 to 61.6 g in the beginning which was close to that of Collins, 51 to 63.5 g. The average group weight when sacrificed ranged from 77.6 to 87.2 g (6 days experiment) compared to 79 to 91 g of Collins (5 days experiment). My lower animal weight mighht be due to more frequent animal room disturbance from the room cleaner. The most obvious decrease in daily weight gain happened on January the 30th. On that day, the room was thoroughly cleaned and thus caused most disturbance to group A and B (Figure 10).

Adjusting the premade spring by expanding or constricting the legs to deliver 20 g of force may result in different remaining force at the end of experiment. The wire tends to lose tension (relaxed). Annealing of

specific antibody compared to 55.3% of cross reactivity Collins used. In other words, the result of LTC_4 assay in my experiment was more close to true LTC_4 value than that of Collins.

C. Results

The non-collagenous protein content of bone samples was used as an estimate of cellular mass to standardize the measurement of LTB_4, LTC_4 and PGE_2 levels. My protein content varied between 4.2% to 11.7%. Benson (1983) reported a consistent 22% protein content while Radulovich (1985) and Collins (1986) reported 9.0-19.4% and 4.0%-7.7% of protein content respectively. The variation in results is probably due to bone sample size, age of the animal and morphology of the osteotomy. Benson used 28-day-old animals, Radulovich, Collins and I used 21-day-old animals to start the experiments. Benson's bone samples varied between 80 and 100 g (20-day experiment), Radulovich's between 10 and 20 g, Collins' between 5 and 10 g (5 day experiment), and my bone samples between 5.2 and 12.7 g.

The higher value of endogenous (control) PGE_2 content, 312 pg/mg protein, compared to 76.8 pg/mg protein of Radulovich and 76.9 pg/mg protein of Collins content may explain the more resorptive histology picture of my control animals. Because there was no sign and symptom of inflammation (no redness, exudates, swelling,

pus, nor lymphocytes infiltration microscopically) found in the animals, the resorptive phenomenon in my control group might be due to rapid sutural remodeling (Elaine Klingler and Delhi Guillaumin 1985). The thick osteoid tissue and sound layer of osteoblasts lining in the ectocranial periosteum showed the rapid bone apposition in the ectocranial surface. While the resorptive surfaces were found only in the endocranial side and part of the sutural area. These appearances were also found in group B, D, E and F. One factor that may affect the normal sutural (calvarial) remodeling is expansion of the brain tissue (Enlow, Facial Growth, Ch 3, 1982). When the expansive force was insulated from affecting the suture by designed osteotomy (Figure 3), the formative activity of the sutural area may have decreased or even became resorptive due to surgical trauma to the area. The other explanation of showing resorptive picture might be Parfitt's (1984) remodeling model. He categorized bone remodeling of adult bone (vs immature bone) into five stages: quiescence, activation, resorption, reversal and formation. The animals might have been in the resorptive dominant ~~stage~~ cycle during the experiment.

The effect of indomethacin was again verified in my work. Group F was 85% depressed in PGE_2 content when compared to group E, while Radulovich had 63% PGE_2 content depression compared to his lower control.

The endogenous LTB_4 content (osteotomy only) 598 pg/mg protein was much lower than that of Collins (1986), 2180 pg/mg protein. If my result was closer to true value, then the applied compressive force did not supress the content of LTB_4 (see Figure 12 group C and D). Mononuclear cells were considered to be precursors of osteoclasts (Tinker et al., 1982; Burger et al, 1984). If LTB_4 acts as a chemoattractor for mononuclear cells to the resorption activated area (Ford-Hutchinson et al, 1980; Malmsten et al., 1980), the presence of LTB_4 would be essential in the area where the resorptive response was activated. And this is consistent with the result in group C and D which had about equal LTB_4 levels with control and had same amount of osteoclasts either in suture or medulary spaces. Because the content of LTB_4 was very low in Collins' pressure group, he questioned whether LTB_4 levels in sutural area under mechanical stress was elevated prior to 5 days. The answer seemed to be negative according to my work. Examination of histology in group B, E and F which were all treated with piriprost and had significant lower LTB_4 contents, few osteoclast could be found on the sutural surfaces even when pressure was applied. This finding is in agreement with Ford-Hutchinson et al., and Malmstem et al., (1980) that LTB_4 is a chemoattractor for mononuclear cells.

Because of the antibody for LTC_4 RIA I used was

The high cellularity and dark stained cuboidal cells found in Collins' experimental groups that received piriprost and tension or pressure have consistantly found in group B and E of my experiment (Plate V and IX, X, XI). This finding was found most strikingly in pressure + piriprost groups (group E of my experimental and group D of Collins). Because those dark stained rounding or cuboidal cells were very like osteoblasts and were existing in the unsmoothed surface, we can conclude that piriprost per se or low LTs content induced this appearance and made the existing of osteoclasts difficult and thus been replaced by the osteoblastic-like cells. The conclusion is also compatible with the findings of Ford-Hutchinson et al (1980) and Malmsten et al (1980).

The swollen and light stained matrix cells found in group F could be due to total or partial blockage of nuclei activities because of the disappearance of the normal stimulation from LTs and PGs to the biosynthetic activities. The nuclei lost their communication with extracellular environments solely because of the necessary metabolites (PGs and LTs) were blocked. In other words to explain this cellular appearance was toxicity of combination use of both 5-lipoxygenase and cyclooxgenase inhibitors.

Other interesting finding in group F were increasing vascularity and bulging endothelial cells of the vessels.

The bulging endothelial cells could be explained by toxicity of the drugs treatment. The increasing of the vascularity could be due to the endogenous defensing mechanism to carry away the toxic drugs from the localized drugs treating area.

D. Clinical significance

The ability of piriprost to exclude the existence of osteoclasts could be valid in treating patients with periodontitis. The problem one may meet will be the instability of piriprost to stay in human gum tissue unless constantly injected. One possible way of delivering the drug constantly and uniformly in amount is the osmotic micropump.

Systemic use of piriprost may be quite complicated. However, it may be valid for some pathologic destructive bone diseases such as Paget's disease and post menopause osteoporosis that are caused by abnormal osteoclastic activities.

SUMMARY AND CONCLUSION

Research in biochemical analysis of bone remodeling has been brought into a new field by Collins (1986). Leukotrienes (LTs), products of 5-lipoxygenase pathway of arachidonic acid, in addition to PGE_2 were quantitatively analyzed in the midsagittal suture of Sprague-Dawley rats to evaluate the relationship between applied stress and the contents of LTs and PGE_2.

LTs are the major inflammatory mediators in human lymphocytes (Dahlen et al 1983). LTB_4 is a potent chemotactic agent toward PMNs, eosinophiles and mononuclear cells (Ford-Hutchinson et al 1982, Malmsten et al 1980). And also, Tinkler et al (1982) and Burger et al (1984) have demonstrated that monocytes and mononuclear phagocytes are most likely the precursors of osteoclasts. Thus my hypothesis is as follow: if osteoclasts originate from monocytes and the LTB_4 attracts monocytes, the blockage of LTs production could also handicap monocytes in migrating towards the remodeling area and/or could prevent monocytes from developing into osteoclasts.

In my study, a sample of 36 weanling female Sprague-Dawley rats was used and divided into 6 groups. Different treatments were given to each group (Table I) to evalute the cellular behavior and sutural LTB_4, LTC_4 and PGE_2 contents in a resorption induced sutural system

versus the untreated formative system. The design was similar to that of Collins (1986) and included in vivo influence of piriprost and compressive force on the midsagittal suture in rats.

At the end of the 6 day experimental period, the suture samples were removed under general anesthesia for both histological and biochemical analyses. The biochemical part was done by a series of lyophilization, pulverization and extracting procedures to process the bone sample for LTB_4, LTC_4 and PGE_2 radioimmunoassay procedures. Levels of non-collagenous proteins were determined for each sample by a variation of the Folin protein assay to standardize the suture samples in terms of active bone tissue. Histological analysis was done by half-strength Karnovsky solution fixation, a series of fixative procedures and finally embedded in maraglass/epon mixture. 1 μm thin sections were cut and stained with Toluidine blue O and Alizarin red S. The significant findings of this study were:

(1) An improved extraction and RIA procedures were used to produce

the following recovery rates for each metabolite.

LTB_4 recovery = 62%

LTC_4 recovery = 80%

PGE_2 recovery = 88%

(2) LTB_4 has the same level of responses between

control, 3 days and 6 days pressure treated groups. In other words, pressure does not increase LTB_4 content in the sutural system in contrast to Collins findings which had significant decrease in LTB_4 content.

(3) LTB_4 levels were significantly reduced in all groups treated with piriprost compared to non-piriprost treated groups; piriprost is potent in inhibiting production of LTB_4.

(4) LTC_4 had slight but not significant elevation in contents between control and pressure (3 days and/or 6 days) treated groups.

(5) Not significant elevation of LTC_4 content was found in group treated with piriprost + control (group B) compared to control (Group A). But this may be considered artifactural.

(6) Piriprost significantly inhibited the formation of LTC_4 in both pressure and pressure + indomethacin (Group E and F) treated groups.

(7) The sutural area showed higher PGE_2 content on the 3rd day of pressure application.

(8) Slight to moderate sutural resorption was mixed with rapid ectocranial periosteal deposition (osteoid tissue) in the sutural areas in control groups.

(9) Piriprost has the strong ability to reduce resorptive system or reverse it to depository

system.

(10) Piriprost has the ability to increase cellularity and makes the cells cuboidal and dark stained to be like osteoblasts especially along sutural invaginations (Plate XI). This phenomenon is most significantly expressed in the group treated with pressure.

(11) Piriprost plus indomethacin treatment may have blocked the necessary mediators for the cells to maintain their biosynthetic activities and has made the cells swollen or damaged.

Findings from this study suggest that certain levels of LTB_4 and LTC_4 contents are necessary for supporting resorptive function. Depressed LTs contents decreases the number of osteoclasts. PGE_2 level was found higher in the initial stage (3rd day compared to 6th day) when applying force in the sutural area.

The future experiments to evaluate the role of LTs in sutural remodeling include: 1) lengthening the 2nd stage (reversing stage by piriprost) of the experiment. 2) testing the effect of exogenous LTs on the sutural growth and remodeling. 3) evaluating other LTs such as LTD_4, LTE_4 and its role on the sutural system or bone remodeling.

TABLE X

EXPERIMENTAL DESIGN DIFFERENCES AMONG SHIAO, COLLINS AND RADULOVICH

CONDITION	RADULOVICH (1985)	COLLINS (1986)	SHIAO (1987)
time of surgery	9:00am-2:00pm	10:00am-3:00pm	1:00-6:00pm
time of sacrifice (biochem.)	12:00am-2:00pm	8:00-10:00pm	8:00-9:00pm
time of sacrifice (histology)	____	3:00-4:00pm	3:00-4:00pm
drug injected	ID	PP	ID + PP
time of inject.	8:00am & 6:00pm	same	same
extraction technique	Hand operated with plastic syringe	same as Radulovich	Alltech vacuum manifold with glass syringe
stresses to the animals	pressure or tension	same as Radulovich	pressure
LTC_4 Ab specificity	———	55.3% X-reaction with LTD_4	11.3% X-reaction with LTD_4
duration of experimental period (days)	5	5	6
metabolite analyzed	cAMP & PGE_2	LTB_4, LTC_4, PGE_2	same as Collins
metabolite column separation used	XAD-2	C-18 microcleanup	same as Collins
Diurnal cycle	11 hrs darkness per day	6:30 am ; pm	6:45 am ; pm

PP = Piriprost

ID = Indomethacin

TABLE XI

MAIN EXP. RESULTS COMPARISON AMONG SHIAO, COLLINS AND RADULOVICH

ITEMS	RADULOVICH* (1985)	COLLINS* (1986)	SHIAO** (1987)
A. Histology			
Pressure + PP	———	Dark stained cuboidal cells dominant, high cellularity (H/E stain) Depository	same as Collins but lighter stained, less cuboidal (TBO+ARS stain) depository and resorptive
Control (Osteotomy)	———		
Pressure + PP + ID	———	———	Swollen and light stained matrix cells swollen endothelial cells
B. Biochemistry (pg/mg protein)			
PGE_2 control	76.8	76.9	312
Pressure	392	96.7	394
tension	287	158	——
Pressure + PP	——	142	350
Pressure + ID	28.6	——	@50
LTB_4 Control	——	2180	598
Control + PP	——	147	142
Pressure + PP	——	524	253
Pressure	——	0	515
LTC_4 Control	——	2630	578
Control + PP	——	2690	1015
Pressure + PP	——	2500	169
Pressure	——	4600	802

* 5 days experiments
** 6 days experiment

PP: Piriprost
ID: Indomethacin
@ : Including PP treated

LITERATURE CITED

Allen, H.L., Wase, A., Bear, W.T.: Indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. Acta Ortho Scand. 51:595-600, 1980.

Bach, M.K., Brashler, J.R., Smith, H.W., Fitzpatrick, F.A., Sun, F.F. and McGuire, J.C: 6.9-Deepoxy-6, 9-(Phenylimino)-6,8-Prostaglandin I, (U-60,257), A New Inhibitor of Leukotriene C and D Synthesis: In Vitro Studies. Prostaglandins 23: 759-771, 1982.

Bach, M.K.: Prospects for Inhibition of Leukotriene Synthesis. Biochem. Pharmac. 33: 515-521, 1984.

Bach, M.K., Brashler, J.R., Peck, R.E. and Morton, D.R.: Leukotriene C Systhetase, a Special Glutathione S-Transferase Properties to the Enzyme and Inhibitor Studies with Special Reference to the Mode of action of U-60,257, a Selective Inhibitor of Leukotriene Synthesis. J. Allergy Clin. Immunol.74: 353-357, 1984.

Bach, M.K., Bowman, B.J., Brashler, J.R., Fitzpatirck, F.A., Griffin, R.L., Johnson, H.G., Major, N.J., McGuire, J.C., McNee, M.L., Richards, I.M., Smith, H.W., Smith, R.J., Speziale, S.C., and Sun, F.F.: Piriprost: A Selective Inhibitor of Leukotriene Synthesis. Adv in Prost, Throm, and Leuk Res 15: 225-227, 1985.

Baron, R., Nefussi, J.R., Duflot-Vignery, A., Lasfargues, J.J., Cohen, F., Puzas, E.: Failure of prostaglandin E to induce local bone resorption in vivo. In: Horten J.E., Tarpley, T.M., Davis, W.R. (eds) Proc mechanisms of localized bone loss Suppl to Calcif Tissue Abstracts, 433-434, 1978.

Benson, J.A.: The Effect of Cyclic 3'5' Adenosine Monophosphate and Cyclic 3'5' Guanosine Monophosphate Upon Sutural Exapnsion by Orthopedic Force. Masters Thesis. Case Western Reserve University, 1983. IADR/AADR Abstract: 1724 J. Dent. Res. 64, 1985.

Binderman, I., Shimshoni, Z. and Somjen, D.: Biochemical Pathways Involved in the Translation of Physical Stimulus into Biological Message. Calcif. Tissue Int. 36: 582-585, 1984.

Borgeat, P., Hamberg, M. and Samuelson, B: Transformation of Arachidonic Acid and homo-μ-linolenic Acid by Rabbit Polymorphonuclear Leukocytes. J. Biol. Chem. 251: 7816, 1976.

Borgeat, P., Nadeau, M., Salari, H., Poubelle, P. and DeLactos, F.: Leukotrienes: Biosynthesis, Metabolism, and Analysis. Advances in Lipid Res. 21: 47-77, 1985.

Burger, E.H., Van Der Meer, J.W., and Nijweide, P.J.: Osteoclast Formation from Mononuclear Phagocytes: Role of Bone-forming Cells. J. Cell Biol. 99: 1901-1906, 1984.

Collins, J.L.: The Effect of Mechanical Stress Upon the Content of Leukotriene B_4, C_4 and PGE_2 in the Midsagittal Suture of the Rat. Master's Thesis. Case Western Reserve University, 1986. IADR/AADR Abstract: 1771. J. Dent. Res. 66, 1987.

Chumbley, A.B., and Tuncay, O.C.: The effect of Indomethacin (an Aspirin-like Drug) on the Rate of Orthodontic Tooth Movement. Am. J. Orthod. 89: 312-314, 1986.

Dahlen, S.E., Hedqvist, P., Hammarstrom, B., Samuelsson, B.: Leukotrienes are potent constrictors of human bronchi. Nature 288: 484-486, 1980.

Dahlen, S., Hedqvist, P., Westlund, P., Granstrom, E., Hammarstrom, S., Lindren, J. and Radmark, O.: Mechanisms of Leukotriene-Induced Contractions of Guinea Pig Airwasy: Leukotriene C_4 has a Potent Direct Action where as Leukotrien B_4 Acts Indirectly. Acta Physiol. Scand. 118:393-403, 1983.

Davidovitch, Z. and Shanfeld, J.L.: Cyclic AMP levels in Alveolar Bone of Orthodontically-Treated Cats. Arch. Oral Biol. 20: 567-574, 1975.

Davidovitch, Z., Finkelson, M.D., Steigman, S., Shanfeld, J.L., Montgomery, P.C., Korostoff, E.: Electric Currents, Bone Remodeling, and Orthodontic Movement. Am. J. Orthod. 77: 33-447, 1980.

Davidovich, Z. and Shanfield, J.L.: Prostaglandin E_2 (PGE_2) Levels in Alveolar Bone Orthodontically-Treated Cats. J. Dent. Res. 59: 362, 1980.

Elves, M.W., Bayley, I., Roylance, P.J.,: The effect of indomethacin upon experimental fracture in the rat. Acta Orth. Scand. 53: 35-41, 1982.

Enlow, D.H.: Handbook of Facial Growth. 2nd Ed., W.B. Saunders Co., Philadelphia, 1982.

Folco, G., Hansson, G. and Granstrom, E.: Leukotriene C_4 Stimulated TxA_2 Formation in Isolated Sensitized Guinea Pig Lungs. Biochem. Pharmac. 30: 2491-2493, 1981.

Ford-Hutchinson, A.W., Bray, M.A., Doig., M.W., Shipley, M.E. and Smith, M.J.: Leukotriene B, a Potent Chemokinetic and Aggregating Substance Released From Polymorphonuclear Leukocytes. Nature 286: 264-265, 1980.

Ford-Hutchinson, A.W.: Leukotrienes: Their Formation and Role As Inflammatory Mediators. Federation Proc. 44: 25-29,.1985.

Feuerstein, N., Bash, J.A., Woody, J.N. and Ramwell, P.W.: Leukotriene C. Stimulates Prostaglandin Release from Rat Peritoneal Macrophages. Biochem. Biophys, Res. Commun. 100: 1085-1090, 1981.

Gilmore, S.K., Whiteson, S.W. and Bowers, D.E., Jr.: A Simple Method Using Alizarin Red S for the Detection of Calcium in Epoxy Resin Embedded Tissue. Stain Technology, Vol. 61, No. 2: 89-92, 1986.

Goodson, J.M., Dewirst, F.E. and Brunetti, A.: Prostaglandin E_2 Levels and Human Periodontal Disease. Prostaglandins 6: 81-85, 1984.

Ham, A.W.: Histology. 7th ed. Philadelphia: J.B. Lippincott, 1974.

Ham, E.A., Soderman, D.D., Zanett, M.E., Dougherty, W.H., McCauley, E. and Kuehl, F.A.: Inhibiton by Prostaglandins of Leukotriene B_4 Release from Activated Neutrophils. Proc. Natl. Acad. Sci. (USA) 80: 4349-4353, 1983.

Hansson, G., Bjorck, T. and Dahlen, B.: Specific Allergen Induces Contraction of Bronchi and Formation of Leukotrienes C_4 D_4 and E_4 in Human Asthmatic Lung. Ad. Prost. Throm. Leuk. Res. 12: 153-159, 1983.

Heersche, J.N.M., Heyboer, M.P.M. and NG, B.: Hormone-Specific Suppression of cAMP Response in Bone In Vitro During Prolonged Incubation with PTH, PGE_2 and Calcitonin. Endocrinology, 103: 333-340, 1978.

Hinrichsen, G.J. and Storey, E.: The Effect of Force on Bone and Bones. Angle Orthod. 38: 155-165, 1968.

Jee, W.S., Ueno, K., Deng, Y.P., Woodbury, D.M.: The Effects of Prostaglandin E_2 in Growing Rats Calcif. Tissue Int. 37: 148-157, 1985.

Johnson, H.G., Chinn, R.A., McNee., M.L., Miller, M.D., Nadel, J.A.: Int. J. Immunopharmacol. 4, 348, 1982.

Kindahl, H. and Granstrom, E.: Methods for Quantitative Estimation of Prostaglandins. Acta Obstet. Gynecol. Scand Suppl. 113: 15-21, 1983.

King, G.J., Thems, S.: Chemical Mediation of Bone Resorption Induced by Tooth Movement in the Rat. Archs. Oral Biol. 24: 811-815, 1979.

Klingler, E. and Guillaumin, D.: Morphological Aspect of the Mid-parietal Suture of the Rat by Scanning Electron Microscopy, Current advances in skeletogenesis, 1983.

Lewis, R.A. and Austin, K.F.: The Biologically Active Leukotrienes Biosynthesis, Metabolism, Receptors, Functions, and Pharmacolgy. J. Clin. Invest. 73: 889-897, 1984.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J.: Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 268, 1951.

Malmsten, C.L., Palmblad, J., Uden, A., Radmark, O., Engstedt, L. and Samuelsson, B.: Leukotriene B_4: A Highly Potent and Stereospecific Factor Stimulating Migration of Polymorphonuclear Leukocytes. Acta Physiol. Scand. 110: 449-451, 1980.

Marcus,R. and Orner, F.: Cyclic AMP Production in Rat Calvaria In Vitro, Interaction of PGs with PTH. Endocrinology, 100: 1570-1578, 1977.

Marfat, A. and Corey, E.J.: Synthesis and Structure Elucidation of Leukotrienes. Adv. Prost. Throm. Leuko. Res. 14: 155, 1985.

Marks, S.C.: The Origin of Osteoclasts: Evidence, Clinical Implications and Investigative Challenges of An Extra-Skeletal Source. J. of Path. 12: 226-256, 1983.

Noyes, F.: Histology of Bone Related to Orthodontic Treatment. Am. J. Orthod. Oral Surg. 28: 760-769, 1942.

Oppenheim, A. Die Veranderungen de Geruebe Isbesondere Des Knochens Bei Der Verschiebung Der Zahne. Osterrich. Vng. Vjschrift, Zahnheilk. 27: 302-358, 1911.

Parfitt, A.M.: The Cellular Basis of Bone Remodeling: The Quantum Concept Re-examined in Light of Recent Advances in the Cell Biology of Bone. Calcif. Tissue Int. 36: S37-S45, 1984.

Powell, W.S.: Rapid Extraction of Oxygenated Metabolites of Arachidonic Acid From Biological Samples Using Octadecylsilyl Silica. Prostaglandins 20: 947-957, 1980.

Radulovich, B.P.: The Effects of Applied Tension and Pressure Upon the Concentrations of Prostaglandin E_2 and Cyclic 3'5' Adneosine Monophosphate in the Midsagittal Suture of the Rat. Masters Thesis. Case Western Reserve University, 1985. IADR/AADR Abstract, 1095, J. Dent. Res. 65. 1986.

Reitan, K: Continuous Bodily Tooth Movembent and its Histological Significance. Acta Odont. Scand. 6: 115, 1947.

Reitan, K: Initial Tissue Behavior During Apical Root Resorption, Angle Orthod. 44:68-82, 1974.

Ro, J.,Sudmann, E., Marton, P.F.: Effect of indomethacin on fracture healing in rats. Acta Ortho. Scand. 47: 588-599, 1976.

Rodan, G.A., Bourret, L.A., Harvey, A. and Mensi, T.: Cyclic AMP and Cyclic GMP: Mediators of the Mechanical Effects on Bone Remodeling. Science 189: 645-469, 1975.

Rosenburg, G., Simmons, D.J., Halberg, F. and Burstein, A.: Trans. Orthoped. Res. Soc. 3: 50-54, 1977.

Samuelsson, B.: The Leukotrienes: An Introduction. Adv. in Prosta. Throm. Leuko. Res. 11: 1-17, 1982.

Samuelsson, B.: Leukotrienes: Mediators of Immediate Hypersensitivity Reactions and Inflammation. Science 220: 568-575, 1983.

Sandy, J.R. and Harris, M.: Prostaglandins and Tooth Movement. Euro. J. Orthod. 6: 175-182, 1984.

Simmons, D.J.: Experimental Design and the Implicatrion of Circadian Skeletal Rhythmicity. Skel. Res. 21: 567-585, 1979.

Simmons, D.J.: Circadian Rhythms in Bone. Metab. Bone Dis. Rel. Res. 2: 137-149, 1980.

Smith, M.J.H.: Biological activities of Leukotriene B_4, Leukotrienes and other Lipoxygenase Products. 283-292, 1982.

Somjen, D., Binderman, I., Berger, E. and Harell, A.: Bone Remodeling Induced by Physical Stress in Prostaglandin E_2 Mediated. Biochem. Biophys. Acta 627: 91-100, 1980.

Songsiridej, V., Buchner, C., Busse, W.: Small airway contribution to antigen-induced contraction of isolated guinea pig (GP) lung parenchymal stripes. J Allergy Clin Immunol 69: 107-111, 1982.

Sutherland, E.W., and Rall, T.W.: The relation of Adenosine 3'5' Monophosphate and Phosphorylase to the Actions of Catecholamines and Other Hormones. Pharmacol. Review 125: 265-299, 1960.

Tinkler, S.M.B., Williams, D.M., Linder, J.E. and Johnson, N.W.: Kinetics of Osteoclast Formation: The Significance of Blood Monocytes as Osteoclast Precursors during 1α-hydroxycholecalciferol-stimulated bone resorption in the Mouse. J. Anat. 137: 335-340., 1983.

Ueno, K., Haba, T., Woodbury, D., Price, P., Anderson, R., Jee, W.S.S. The effects of Prostaglandin E_2 in rapidly growing rats: depressed longitudinal and radial growth and increased metaphyseal hard tissue mass. Metab Bone Dis and Rel Res. 79-86, 1985.

Vanderhoek, J.K., Bryant, R.W. and Bailey, J.M.: Regulation of Leukocyte and Platelet Lipoxygenases by Hydroxyeicosanoids. Biochem. Pharm. 31: 3463-3467, 1982.

White, R.K., and Montgomery, S.: Inflammatory Mediators - A Review. Oral Surg. Oral Med. Oral Pathol. 61: 514-518, 1986.

Willoughby, D.A.: The Inflammatory Response. J. Dent. Res. 51: 226-227, 1972.

Yamasaki, K., Miura, F. and Suda, T.: Prostaglandin as a Mediator of Bone Resorption Induced by Experimental Tooth Movement in Rats. J. Dent. Res. 59: 1635,-1642, 1980.

Yamasaki, K., Shibata, Y. and Fukuhara, T.: Basic Biological Sciences: The Effect of Prostaglandins on Experimental Tooth Movement in Monkeys (Macaca Fuscata). J. Dent. Res. 61: 1444-1446, 1982.

Yamasaki, K.: The role of Cyclic AMP, Calcium , and Prostaglandins in the Induction of Osteclastic Bone Resorption Associated with Experimental Tooth Movement. J. Dent. Res. 62: 877-881, 1983.

Yamasaki, K., Shibata, Y., Imai, S., Tani, Y., Shibasaki, Y. and Fukuhara, T.: Clinical Application of Prostaglandin E_1 (PGE_1) upon Orthodontic Tooth Movement. Am. J. Orthod. 85: 508-518, 1984.

Yu, J., Wells, H., Ryan, Jr., W.J. and Lloyd, W.S.: Effects of Prostaglandins and Other Drugs on the Cyclic AMP Content of Cultured Bone Cells. Prostaglandins 12: 502-510, 1976.

52 87 365CS2 0 104 7 TH 6570