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The effect of duration and magnitude of tensile mechanical forces on sutural tissue *in vivo*

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SUMMARY Orthodontic springs were placed across the interparietal suture in twenty 30-day-old male Wistar rats, in order to study the effect of tensile forces on the initial biological response of sutural tissues. Five groups of different force duration and magnitude were used: a 6 hours (h), 0 mN group; 6 h and 24 h, 50 mN groups; and 6 h and 24 h, 100 mN groups. One group of four animals served as a control.

The animals were injected with tritiated proline 3 hours prior to the end of the experiment. Undecalcified 5 μm sections were used for (enzyme) histology and autoradiography in order to quantify several morphometric variables. The data were analysed with multivariate analysis of variance and contrast calculations. Application of the springs led to significant sutural widening within 6 hours. The concentration of fibroblasts in the suture also increased significantly within 6 hours. The volume of the suture and the incorporation of ^{3}H-proline in the fibrous part of the suture and in the osteoid along the sutural bony edges were significantly increased after 24 hours of force. In general, force duration had a greater impact on histological events than force magnitude.

Introduction

Treatment techniques aiming at the modification of growth in the orofacial region are frequently used in orthodontics and dentofacial orthopaedics. The objective is to obtain more harmonious skeletal and dental relationships.

Shape, size, and spatial relationships of the facial bones can be altered in growing subjects by forces exerted by various kinds of orthopaedic appliances. A major part of the remodelling processes necessary for the adaptation to the altered functional environment takes place in the sutures which connect the various facial and cranial bones. These sutures are the sites where (bone) remodelling takes place when the craniofacial complex is subjected to tensile, compressive, or shearing mechanical forces (Hinrichsen and Storey, 1968; Murray and McCleall, 1971; Ten Cate *et al.*, 1977; Jackson *et al.*, 1979; Meikle *et al.*, 1979; Nanda and Hickory, 1984).

A number of studies have provided histological descriptions of suture response to tension (Hinrichsen and Storey, 1968; Murray and McCleall, 1971; Ten Cate *et al.*, 1977; Jackson *et al.*, 1979; Nanda and Hickory, 1984). Those studies that have quantified the suture response to tension, have focused on the anabolic aspects of remodelling, such as collagen synthesis (Meikle *et al.*, 1979, 1984; Yen *et al.*, 1984; Miyawaki and Forbes, 1987) and cell proliferation (Hickory and Nanda, 1987). Remodelling of bone, however, also implies resorption. No attempts have so far been made to quantify the osteoclastic activity in cranial bones under tension.

In most *in vivo* studies, the applied forces ranged from 500 mN ('low') to 2500 mN ('heavy'), depending on the type of animal and suture under investigation. Sometimes the applied forces were up to 5000 mN (Nanda and Hickory, 1984). In *in vitro* studies, the forces were smaller. Meikle and colleagues (1979, 1984) used forces of 200–300 mN, while Hickory and Nanda (1987) used forces of less than 10 mN.

The relationship between the character of applied mechanical force variables, such as magnitude, direction, constancy and duration, and the character and intensity of the biological response in the sutural tissues has been investigated in only a limited number of studies, such as the combined *in vivo/in vitro* study by Miyawaki and Forbes (1987), and the *in vitro* study by Hickory and Nanda (1987). Comparable *in vivo*

dose–response experiments, however, are lacking. How mechanical forces are translated into biological, i.e. cellular responses is largely unknown (Wagemans *et al.*, 1988), although some steps in this process have been elucidated (Binderman *et al.*, 1988).

The aim of this study was to investigate the cellular and biochemical *in vivo* response of the rat interparietal suture to small tensile forces of different magnitudes and durations.

To this end, the proliferation of fibroblasts and the incorporation of ^{3}H-proline in bone matrix and tissue fibres were chosen as parameters for (bone) formation. ^{3}H-proline is a marker for protein synthesis, the major part being collagenous proteins (Yen *et al.*, 1984). The number and distribution of osteoclasts, as an indication for bone resorptive activity, were studied for their enzyme histology.

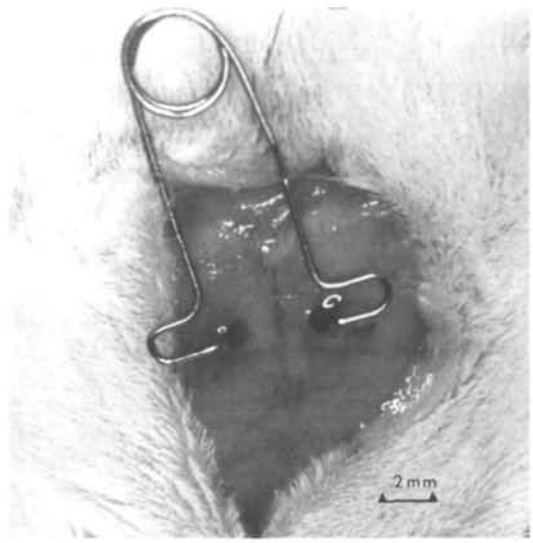

Figure 1 Orthodontic spring (0.014 steel) *in situ*. Posterior side of animal above.

Materials and methods

Animals

Twenty-four 30-day-old male Wistar rats from the Wag-Rij strain (TNO Proefdierenlaboratorium Rijswijk, The Netherlands) were used. The animals were kept under standard laboratory conditions and were allowed food and drink *ad lib* (standard laboratory rat chow, Hope Farms).

The animals were divided into six groups of four rats each: a control group, a group wearing passive, i.e. inactivated springs for 6 hours (h), two groups wearing 50 mN activated springs for 6 and 24 h, and two groups wearing 100 mN activated springs for 6 and 24 h.

Three hours prior to the end of the experiment, the animals were injected i.p. with a dose of 3 μCi ^{3}H-proline (600 μCi/ml saline) per g body weight. After the experimental period, the animals were killed by ether intoxication.

Operation technique

The animals were anaesthesized with an i.m. injection of 0.03–0.05 ml Hypnorm®, weighed, and shaven in the operational area. An anteroposteriorly directed incision was made to reveal the skull. In the parietal bones, on each side of the interparietal suture a hole was drilled using a 0.9 mm diameter round low-speed burr cooled with saline. The holes were located halfway the frontal and dorsal ends of the suture. The outer edges were about 4 mm apart. The precise distance between the outer edges of the holes after preparation was measured twice using a measuring gauge with an accuracy of 0.1 mm. At this stage a spring was inserted in the holes, with the helical end directed posteriorly (Fig. 1). After application of the spring the skin was sutured with 6 × 0 silk.

Spring design

The design of the springs allowed for an easy, self-locking application in the holes.

The springs were manufactured from two different materials, depending on the required force delivery. 0.014 Stainless steel orthodontic wire was used for 100 mN activated springs. 0.016 Titanium molybdenum alloy (TMA) wire (ORMCO) was used for 50 mN springs, because of its better load-deflection properties.

Load-deflection rates were 25 mN/mm for the steel springs and 15 mN/mm for the TMA springs. Both types of wire used twice as passive, 0 mN springs in the sham-operated animals.

Spring calibration was performed with 5 and 10 g weights by holding the spring vertically in a plier at the lower leg and hanging the weight on the upper one. The springs were readjusted until the distance between the legs was equal to the precise distance between the outer edges of the holes in the parietal bones.

Histology and autoradiography

Parts of the skulls containing the parietal bones and 3–4-mm wide margin were carefully removed. The anterior halves of the cranial bone pieces were fixed overnight in a paraformaldehyde solution and then dehydrated. Following embedding in Technovit®, transverse undecalcified 5-μm thick sections were made with a Jung K microtome.

From each animal, twenty sections were stained with a Goldner-stain, and twenty with haematoxylin. The latter were reacted for tartrate-resistant acid phosphatase (TRAcP). TRAcP has widely been used as a marker to identify osteoclasts (Hammarstrom *et al.*, 1971; Minkin, 1982; Baron *et al.*, 1986; Van de Wijngaert and Burger, 1986).

The TRAcP-stained sections were used for autoradiography. The slides with sections were dipped in Ilford K-2® emulsion and developed in Ilford Phenisol® after 3 weeks exposure in total darkness.

Morphometrics

Every fourth TRAcP-stained section was traced with a drawing prism at a magnification of $\times 250$, drawing the sutural bony edges, the vessels, and cell-free areas. Since sutures exhibit a variable morphology, a construction system was used to establish the endo- and ectocranial limits of the fibrous sutural area (Fig. 2).

With a digitizer and computer both endo- and ectocranial widths were measured twice and averaged to obtained the sutural width. If the second measurement differed more than 2 per cent from the first one, both were performed again.

The area between the sutural bony edges to the left and right sides of the suture, and the lines described above was measured in an analogous way. The areas of blood vessels and cell-free zones (i.e. areas with small ruptures and artefacts) were recorded separately. These areas were subtracted from the total area to obtain the net cellular area. This net cellular area was used to estimate the total number of fibroblasts in the section.

Fibroblasts were defined as spindle-shaped cells with abundant basophilic cytoplasm (Cormack, 1987). The concentration of fibroblasts was established by counting an area of 10 squares (13,840 μm^2 in a Zeiss integrating eyepiece at a magnification of $\times 250$. The squares had to be located over cellular areas, not containing blood vessels or ruptures in the suture. Together with the net cellular area measurements, the total number of fibroblasts present in each section could be estimated. This estimate was used as an indicator of the proliferative activity in the suture.

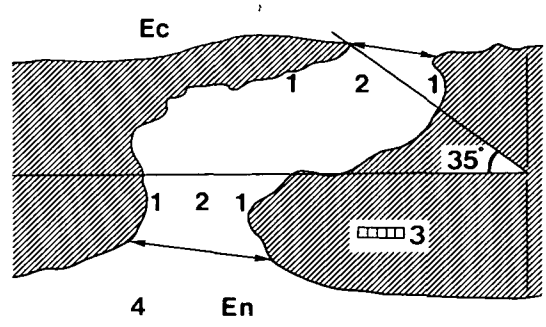

Figure 2. Tracing of transverse section and construction system used to establish suture limits. The points where a 35° angle to the long transverse axis of the section touches upon the bone are considered to be the sutural limits. A 10×10 ocular counting grid was used for grain counting (magnification $\times 1000$) at four places of the sutural bony edge (1), at two places in the fibrous sutural tissue (2), and at the bone (3). The five squares indicate the size of the counted area ($5 \times 92\ \mu m^2$). The background (4) was assessed by counting grains in 10 squares, the mean value was then considered to be the background for that specific section.

TRAcP-positive cell profiles were classified into the following size groups:

(1) large, multinucleated red or rose cell profiles (the 'typical' osteoclast);

(2) medium, single nucleated red or rose cell profiles, or cell profiles of the same size having a red colour, but without a nucleus.

These larger size groups were considered to be osteoclast profiles. All other small red or rose structures were not counted.

Subsequently, the location of the osteoclast profiles in each size group was considered. The first category of osteoclast profiles was lying close to the bone surface. The second category was located between the sutural fibres. An intermediate category was made for osteoclast profiles located at a distance not larger than their own diameter from the bone, but without touching it.

Autoradiographic grain counting was performed in various regions of the sections at a magnification of $\times 1000$ (Fig. 2).

In each section, five squares were counted in each of the following regions.

1. bone limit–the region directly touching the mineralized bone and 10 μm wide (partly consisting of osteoid);
2. Suture—(middle fibrous part), at more than 25 μm from the sutural bony edge;
3. Bone—at more than 25 μm from the sutural bony edge;

The background was assessed counting 10 squares.

Statistics

The morphometric data was analysed by means of multivariate analysis of variance (SPSSX MANOVA) including contrast calculations (Nie, 1983).

Results

The operative techniques were easy to perform and gave little discomfort to the animals. After the anaesthesia ended, no difference in behaviour between experimental and control animals could be observed. Application of the tensile forces led to a widening of the suture (Fig. 4), without visible mechanical trauma, signs of inflammation, pyknosis, or necrosis (Figs 3 and 4).

^{3}H-proline incorporation, manifest by the presence of autoradiographic grains, is predominantly localized at the endocranial surfaces of the parietal bones and in the marrow cavities (Figs 5 and 6). These specific surfaces are covered with osteoid seams, which can clearly be distinguished in the Goldner-stained sections. Following force application, the density of autoradiographic grains is visibly raised at the sutural bony edges (Fig. 6).

The mean values and standard deviations of the morphometric variables in the control group and experimental groups are listed in Tables 1A, 2A, and 3A. The morphometric data were analyed by MANOVA. Significant differences in morphometric variables between the various groups with force duration, force magnitude, and force duration by force magnitude are listed in Tables 1B, 2B, and 3B. Those morphometric variables not mentioned in Tables 1B, 2B, and 3B did not show any significant differences.

Significant differences with force duration between the various groups were found for the width and area of the suture, the net cellular area, the number and concentration of the sutural fibroblasts, and the number of autoradiographic grains in the osteoid and in the fibrous area of the suture. Significant differences with force magnitude between the groups were found in the number of sutural fibroblasts.

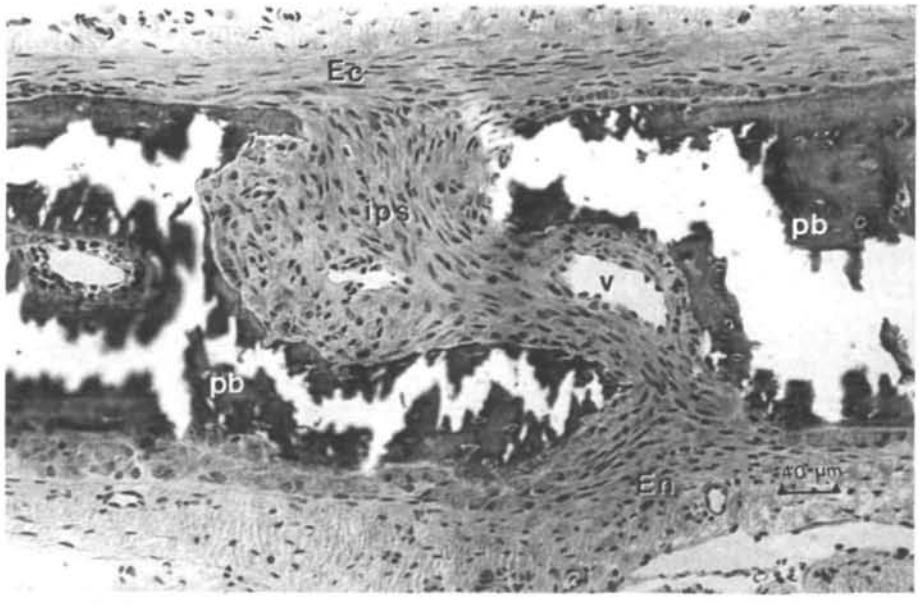

Figure 3 Goldner-stained 5 μm transverse section of interparietal suture of control group animal. (Magnification, ×218.)

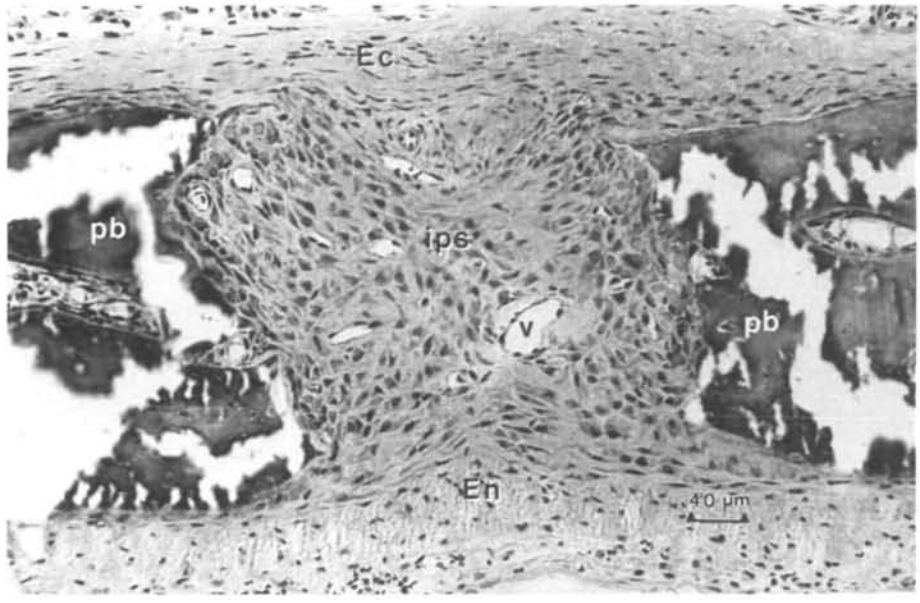

Figure 4 Goldner-stained 5 μm transverse section of interparietal suture of 50 mN, 24 h animal. (Magnification, $\times 218$).

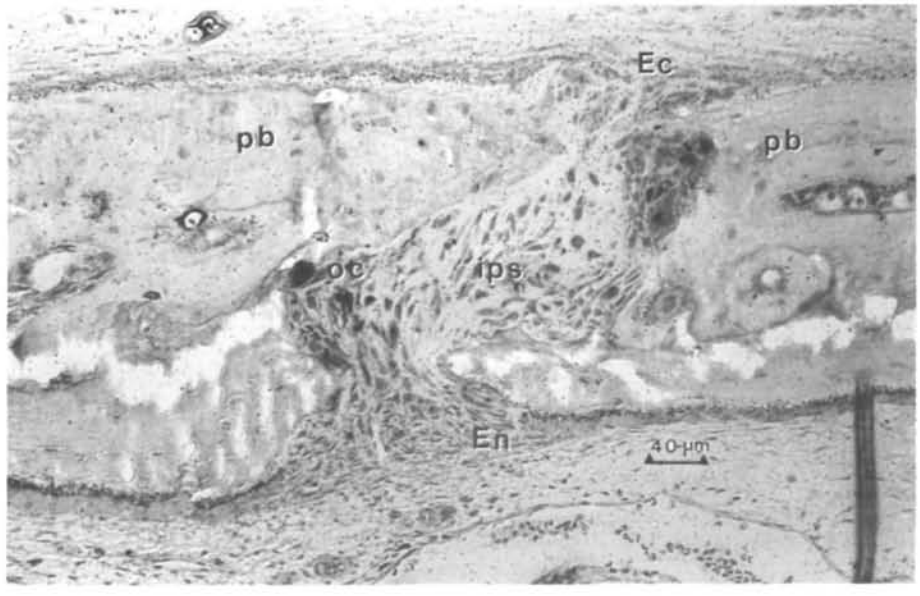

Figure 5 TRAcP-stained transverse section of interparietal suture of control animal. Osteoclast profiles (dark structures) are located directly to the sutural bony edge. Autoradiographic grains are scarce in the sutural area. (Magnification, $\times 218$).

For a number of variables, the effect of force duration in the 50 mN groups was significantly different from that in the 100 mN groups (interaction effect force duration by force magnitude). These variables are the sutural area and net cellular area, the number and concentration of sutural fibroblasts, and the number of osteoclasts at or close to the sutural bony edge (Tables 1B, 2B, and 3B). No significant differences in any of the morphometric variables were found

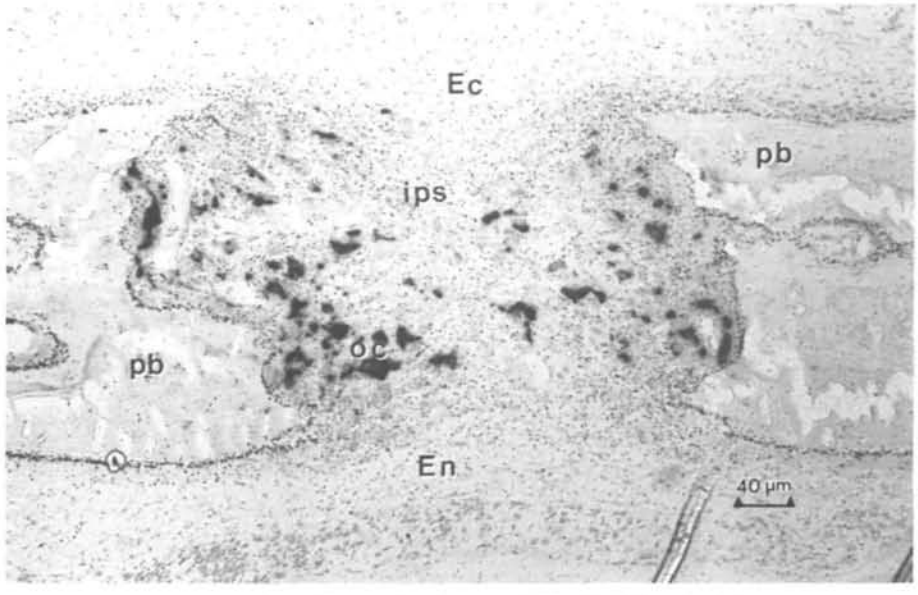

Figure 6 TRAcP-stained transverse section of interparietal suture of 50 mN, 24 h animal. Osteoclast profiles are located in between the fibrous sutural tissue. Autoradiographic grains are concentrated at the sutural bony edges. (Magnification, × 218.)

between the various sections within any animal, implying that the histological events were evenly distributed through the suture. If morphometric variables showed significant main or interaction effects (Tables 1B, 2B, and 3B) a number of hypotheses about differences between groups were tested by contrast calculations. In the case of a significant force duration effect, force duration within force magnitude contrasts were calculated. If the force magnitude effect was significant, force magnitude within force duration contrasts were calculated. In the case of a significant force duration by force magnitude interaction effect, both types of contrast were calculated. The results of these contrasts are given in Table 4. The contrast calculations show that the sutural width and the concentration of sutural fibroblasts are significantly increased after 6 hours of spring application, be it active or inactive. The concentration of the fibroblasts is highest after 24 hours of 100 mN force action.

The width, area and net cellular area of the suture, and the number of autoradiographic grains in the osteoid and the fibrous sutural region significantly increased after 24 hours of tensile forces. The sutural area is significantly greater after application of a 100 mN spring. The number of fibroblasts in the suture is significantly increased after 24 hours of 100 mN force action.

The counts of the osteoclast population (Table 2A) indicated that the total number of osteoclast

Table 1A Means (and standard deviations) of morphometric variables per group of four animals.

Morphometric variables	Control	0 mN, 6 h	50 mN, 6 h	100 mN, 6 h	50 mN, 24 h	100 mN, 24 h
Width	159.9 (50.7)	191.2 (28.6)	194.8 (24.7)	256.8 (25.5)	331.8 (67.9)	399.6 (16.4)
Area	41.1 (10.8)	41.8 (9.7)	59.1 (9.5)	48.7 (13.3)	77.9 (10.6)	110.5 (30.1)
Bloodvessels	0.9 (0.2)	2.2 (1.4)	1.9 (0.5)	1.3 (1.1)	1.6 (1.7)	3.1 (1.7)
Cellfree area	2.1 (2.7)	0.7 (0.6)	1.7 (0.5)	2.0 (0.2)	2.2 (1.8)	1.1 (0.8)
Net cell area	38.1 (10.2)	39.0 (9.0)	55.4 (8.8)	45.4 (12.7)	74.1 (10.1)	106.4 (30.5)
Fibconc	56.3 (6.4)	61.9 (2.6)	79.6 (3.5)	80.3 (5.1)	66.9 (1.5)	80.0 (7.2)
Totfib	153.0 (38.7)	174.7 (41.4)	317.6 (39.6)	265.4 (82.3)	357.0 (41.2)	620.9 (206.4)

Table 1B Results of the MANOVA calculations on the variables mentioned in Table 1A. No other morphometric variables showed any significant effect.

	Effect					
	Force duration		Force magnitude		Force duration by force magnitude	
Morphometric variable	*F*-ratio	$P \leq$	*F*-ratio	$P \leq$	*F*-ratio	$P \leq$
Width	21.28	0.000				
Area	9.28	0.002			7.41	0.014
Net cell area	9.36	0.002			7.39	0.014
Fibconc	7.40	0.004	18.4	0.000	6.5	0.020
Tofib	7.80	0.004			10.73	0.004

Key to Table 1
Width: mean ecto- and endocranial sutural width (μm).
Area: sutural area (10^3 μm^2)
Bloodvessels: area of blood vessels (10^3 μm^2).
Cell-free area: area of artefacts (10^3 μm^2).
Net cell area: area minus cell-free and vessel areas (10^3 μm^2).
Fibconc: number of fibroblasts on 13840 μm^2.
Totfib: number of fibroblasts per section (Fibconc* Net area/13840).

profiles present in the suture was not significantly different among the groups during the experimental period. The total number of osteoclast profiles located against the sutural bony edge showed a significant difference among the groups (the interaction effect force duration by force magnitude, Table 2B). However, no significant models were obtained from contrast calculations.

The numbers of autoradiographic grains in the endocranial and ectocranial osteoid of the suture were analysed simultaneously in a separate multivariate analysis of variance. Significant differences with force duration between the groups were found for the number of autoradiographic grains in the ectocranial and endocranial osteoid of the suture ($F = 4.78$, $P < 0.05$ and $F = 3.92$, $P < 0.01$, respectively). The contrast calculations show that the number of autoradiographic grains in the endocranial osteoid is significantly increased between 6 and 24 hours of 100 mN force action ($F = 7.56$, $P < 0.05$), whereas the number of grains in the ectocranial osteoid is significantly increased between 6 and 24 hours of 50 mN force action ($F = 4.48$, $P < 0.05$).

Table 2A Means (and standard deviations) of morphometric variables per group of four animals.

Morphometric variables	Control	0 mN, 6 h	50 mN, 6 h	100 mN, 6 h	50 mN, 24 h	100 mN, 24 h
Ostlargebone	1.4 (0.9)	2.5 (0.5)	2.1 (2.4)	2.6 (1.3)	0.8 (0.6)	2.1 (1.7)
Ostmedbone	1.3 (1.2)	1.3 (0.8)	1.1 (0.5)	1.0 (0.5)	1.4 (1.1)	1.2 (0.6)
Ostbone	2.6 (2.0)	3.8 (1.0)	3.2 (2.9)	3.6 (1.7)	2.3 (1.3)	3.3 (2.2)
Ostlargeinter	0.3 (0.2)	0.8 (0.6)	1.0 (1.1)	0.9 (0.4)	0.6 (0.5)	0.8 (0.5)
Ostmedinter	1.2 (0.6)	1.1 (0.3)	2.1 (0.4)	1.3 (0.5)	1.1 (0.3)	2.2 (0.4)
Ostinter	1.4 (0.7)	1.9 (0.5)	3.1 (1.0)	2.3 (0.5)	1.8 (0.5)	2.9 (0.8)
Ostlargefree	0.9 (0.5)	1.1 (0.9)	0.5 (1.0)	0.7 (0.6)	1.5 (1.5)	0.9 (0.6)
Ostmedfree	3.6 (1.6)	2.3 (1.0)	2.6 (0.9)	4.0 (2.6)	4.1 (2.6)	2.9 (0.6)
Ostfree	4.4 (1.9)	3.3 (1.8)	3.1 (1.6)	4.6 (3.1)	5.6 (3.8)	3.8 (1.2)
Ostlarge	2.6 (0.8)	4.4 (1.4)	3.6 (2.2)	4.2 (1.5)	2.9 (0.8)	3.7 (2.3)
Ostmedium	5.9 (1.7)	4.6 (1.2)	5.8 (0.8)	6.3 (3.2)	6.7 (1.9)	6.3 (1.2)
Osttotal	8.5 (2.3)	9.0 (2.2)	9.3 (2.8)	10.4 (4.4)	9.6 (2.6)	10.0 (3.2)
Ostbone + inter	4.1 (2.6)	5.7 (1.2)	6.3 (2.2)	5.8 (2.1)	4.0 (1.7)	6.2 (2.3)
Ostinter + free	5.9 (1.3)	5.3 (2.2)	6.2 (2.2)	6.9 (3.2)	7.4 (3.6)	6.8 (2.0)

Table 2B Result of the MANOVA calculations on the variables mentioned in Table 2A. No other morphometric variables showed any significant effect

Effect	Force duration by force magnitude	
Morphometric variable	*F*-ratio	$P \leq$
Ostbone+inter	6.43	0.026

Key to Table 2
Ostlargebone: number of large osteoclasts lying against sutural bony edge
Ostmedbone: number of medium osteoclasts lying against sutural bony edge.
Ostbone: total number of osteoclasts lying against sutural bony edge.
Ostlargeinter: number of large osteoclasts in intermediate position.
Ostmedinter: number of medium osteoclasts in intermediate position.
Ostinter: total osteoclasts in intermediate position.
Ostlarge-free: number of large osteoclasts in fibrous zone.
Ostmed-free: number of medium osteoclasts in fibrous zone.
Ost-free: total osteoclasts in fibrous zone.
Ostlarge: total large osteoclasts.
Ostmed: total medium osteoclasts.
Osttotal: total osteoclasts.
Ostbone+inter: osteoclasts at the bone plus intermediate position.
Ostinter+free: osteoclasts in intermediate position plus in fibrous zone.

Discussion

This study demonstrates that small forces of 50 or 100 mN induce a variety of biological changes in the rat interparietal sutural area within 6–24 hours. Since relatively small forces of physiological level were used in this study, no mechanical trauma, signs of inflammation, pyknosis, or necrosis could be observed at the light microscopic level. In other sutural expansion studies the magnitude of the forces used induced a traumatic sutural tissue response (Ten Cate *et al.*, 1977).

Table 3B Results of the MANOVA calculations on the variables mentioned in Table 3A. No other morphometric variables showed any significant effect.

Effect	Force duration	
Morphometric variable	*F*-ratio	$P \leq$
Arosteoid	3.53	0.05
Arfibres	4.65	0.024

Key to Table 3.
Arosteoid: autor. grains on 92 μm^2 in osteoid area.
Arfibres: autor. grains on 92 μm^2 in fibrous sutural region.
Arbone: autor. grains on 92 μm^2 in bone area.
Arostecto: autor. grains on 92 μm^2 in ectocranial osteoid area.
Arostendo: autor. grains on 92 μm^2 in endocranial osteoid area.

The initial reaction of the sutural tissue to application of tensile mechanical force is partly mechanical in nature: the increase in width of the suture is established as a more or less immediate reaction to the application of tensile force, as the bones are being pulled apart. This was described by Hinrichsen and Storey (1968).

The concentration of fibroblasts is increased after 6 hours of spring application. This is in accordance with the observation of Smith and Roberts (1980), who found an increase of ^{3}H-thymidine labelled cells in the rat periodontal membrane after orthodontic tooth movement within 90 minutes. Hickory and Nanda (1987) also suggested a rapid proliferative response. The sensitivity of the response mechanism even causes an effect in the passive spring group. This may be caused by the loose attachment of the springs, which might move a little in the holes in the parietal bones, thereby inducing microforces. The same phenomenon was observed in rabbits wearing passive springs fixed to their incisors (Van de Velde *et al.*, 1988). The total number of

Table 3A Means (and standard deviations) of morphometric variables per group of four animals.

Morphometric variables	Control	0 mN, 6 h	50 mN, 6 h	100 mN, 6 h	50 mN, 24 h	100 mN, 24 h
Arosteoid	7.5 (4.1)	7.4 (2.9)	7.2 (5.5)	13.5 (6.8)	15.1 (4.1)	19.0 (3.4)
Arfibres	3.5 (2.2)	4.3 (1.0)	3.3 (0.7)	4.6 (1.1)	5.6 (1.8)	6.5 (1.0)
Arbone	2.4 (1.5)	0.4 (0.2)	−0.3 (1.2)	0.5 (0.6)	1.2 (0.2)	1.7 (1.2)
Arostecto	7.2 (2.9)	5.7 (4.0)	5.7 (6.2)	12.2 (10.0)	14.2 (3.9)	16.0 (1.9)
Arostendo	7.9 (5.5)	9.0 (2.9)	8.6 (7.8)	14.7 (5.9)	15.9 (4.3)	22.0 (5.1)

Table 4 The results of the contrast calculations are summarized in the following models, where μ_c indicates the hypothetical mean for that variable in the control population, etc.

Morphometric variables	Models derived from contrast calculations										
Width	μ_c	<	$\mu_{0,6}$	=	$\mu_{50,6}$	=	$\mu_{100,6}$	<	$\mu_{50,24}$	=	$\mu_{100,24}$
Area	μ_c	=	$\mu_{0,6}$	=	$\mu_{50,6}$	=	$\mu_{100,6}$	<	$\mu_{50,24}$	<	$\mu_{100,24}$
Net cell area	μ_c	=	$\mu_{0,6}$	=	$\mu_{50,6}$	=	$\mu_{100,6}$	<	$\mu_{50,24}$	=	$\mu_{100,24}$
Fibconc	μ_c	<	$\mu_{0,6}$	<	$\mu_{50,24}$	<	$\mu_{50,6}$	=	$\mu_{100,6}$	<	$\mu_{100,24}$
Totfib	μ_c	=	$\mu_{0,6}$	=	$\mu_{50,6}$	=	$\mu_{100,6}$	=	$\mu_{50,24}$	<	$\mu_{100,24}$
Arosteoid	μ_c	=	$\mu_{0,6}$	=	$\mu_{50,6}$	=	$\mu_{100,6}$	<	$\mu_{50,24}$	=	$\mu_{100,24}$
Arfibres	μ_c	=	$\mu_{0,6}$	=	$\mu_{50,6}$	=	$\mu_{100,6}$	<	$\mu_{50,24}$	=	$\mu_{100,24}$

fibroblasts in the present study is raised along with the increases in sutural width and area. This also reflects the rapid response capacities of the sutural tissue to changing environmental circumstances. The findings indicate that force duration is a more important factor in the character of the initial response of the fibroblastic population than the magnitude of the applied force, although there is a force duration by force magnitude interaction in several of the variables studied. Bearing in mind, however, that this study is cross-sectional, any interpretations of changes with time have to be made with reserve.

Miyawaki and Forbes (1987), however, using much heavier forces than in the present study, suggested that the response of the connective tissue correlated positively with the magnitude of the applied force. After 24 hours of 500–700 mN ('light') spring force, very little widening of the suture and localized areas of stretched collagenous fibres were observed, as well as few mitotic figures. After 24 hours of 2000–2500 mN ('heavily') spring force, greater stretching and abundant mitotic figures were present.

After 24 hours of force application, small changes in the osteoclastic population are visible. In some of the animals, there is a diminished ratio of osteoclast profiles at the bone surface versus 'free' osteoclast profiles. This may indicate a diminished need for bone resorption, in accordance with the generally accepted theory that tensile forces acting on bone cause net bone formation (Reitan, 1975).

Osteoclasts were identified by their strong reactivity to TRAcP staining. Only large and medium sized cells were counted in which the entire cytoplasm reacted. Also the location of the TRAcP positive cells was taken into consideration. This was done, because it is known that osteoblasts and osteocytes can also be stained with tartrate-resistant acid phosphatase. The reactivity in these cells, however, is much weaker than in osteoclasts and characterized by a punctate cytoplasmic reactivity (Bianco *et al.*, 1988).

The increase in ^{3}H-proline incorporation in the sutural area is a sign of increased fibre synthesis and of osteoid deposition by increased osteoblastic activity along the sutural bony edges. This is most pronounced after 24 h of force application. In contrast to the 50 mN force, the 100 mN force is capable of inducing a higher incorporation along the sutural bony edges within 6 h, although the effect is quite variable. These findings are in line with the results of Meikle *et al.* (1979), who reported a two-fold increase of ^{3}H-proline incorporation after 6 hours of mechanical stress application to rabbit cranial sutures *in vitro* and a three-fold increase after 24 hours.

One-day application of tensile forces to the rat interparietal suture affect fibroblasts, osteoblasts, and osteoclasts. The changes in the osteoclastic population, however, are small and hard to interpret. Significant osteoclastic changes may perhaps occur after longer periods of force application.

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