Experimental Studies on the Interrelations of Condylar Growth and Alveolar Bone Formation III. Response to Relief of Occlusion in Aged Rats

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Introduction

Relief of occlusion in teeth with unlimited growth, e.g., rat incisors, has been studied repeatedly. It resulted in a 2-3 fold acceleration of proliferation at the growing end of the tooth which persisted for months provided that the tooth was kept out of occlusion by repeated cutting of the erupted portion.[1] Release of the occlusion of molars in rats was studied by Schneider and Meyer[2] and by Cimasoni and Becks[3] and shown to result in a similar acceleration of eruption as in the case of the incisors. However, this was not brought about by accelerated growth of the tooth itself, but by increased alveolar bone formation at the interradicular crest and fundus and only a slight response of apical cementum. The data given by Cimasoni and Becks are compatible with the conclusion that accelerated alveolar bone formation had persisted for sixty days in molars kept out of occlusion for this length of time. Schneider and Meyer therefore suggested that removal of the inhibitory masticatory forces permits alveolar bone to manifest its full growth potential.

Since relief of occlusion has not been studied in older animals, the present study was undertaken to test the growth potential of alveolar bone in rats of old age.

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Material and Methods

Thirty male albino rats (retired breeders) of the Charles River strain were used in this study. Twenty rats, 450 days of age and ranging between 534 and 833 g, served as experimental animals (group I), and ten, 560 days of age and ranging between 520 and 784 g, as control animals (group II).

Control and experimental animals were anesthetized and given an intravenous injection of lead acetate at a dosage of 4 mg/kg. Occlusion of the three right mandibular molars was relieved in the experimental animals by grinding the antagonists to below the level of the gingival margin. Measurements in representative teeth showed that this amounted to a reduction of crown height by about 0.5 mm. It was estimated that in a sixteen day experimental period occlusion would not be reestablished. This assumption was verified by means of a second injection of lead acetate given to the experimental animals nine days later. All animals were killed sixteen days after the first lead injection.

Surgical procedures The animals were anesthetized with a 3% solution of sodium pentobarbital injected intraperitoneally at a dosage of 30 mg/kg. Lead acetate was injected into the femoral vein. The right maxillary molars were rapidly ground, using a round diamond stone mounted in a handpiece on a dental engine, to below the level of the gingival margins. Some bleeding and wide exposure of the pulp chambers ensued. The animals were lethargic for the remainder of the day, but returned to their diet of purina rat pellets and water within a day or two. Although no sterile procedure had been used, there was surprisingly little periapical involvement at postmortem examination of twelve of the ground molars. The antagonists of the ground teeth, i.e., the right mandibular molars, were studied for the response to relief of occlusion. The molars of the left side of the operated animals and of both sides of the unoperated animals served as controls. All animals were weighed every second day.

Preparation of specimens At the end of the experimental period the animals were killed with ether and decapitated. The head was cut midsagittally, the mandibular halves disarticulated, and the soft tissues removed. The mandibles were fixed in 10% neutral formalin, washed, and decalcified in hydrochloric acid saturated with hydrogen sulfide. Decalcification was complete within three to five days. Following this, the specimens were washed overnight in running tap water and the molar-bearing segments prepared for embedding in gelatine and cut on a freezing microtome set to a thickness of 15 μ. The plane of sectioning went through the centers of the distal roots of the first and second mandibular molars. To enhance the lead sulfide markings, the sections were treated with gold chloride following the method of Seiton.[4]

Photography Microphotographs of representative sections of each specimen were made and printed at final magnifications of 28 x and 117 or 381 x. The lower magnification gave an overall view of the molar region (Figure 1).

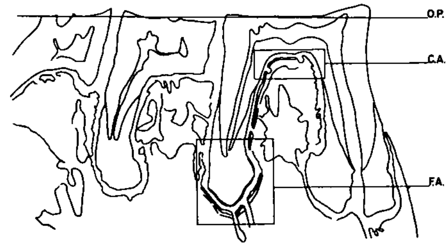

Fig. 1 Outline drawing of a sagittal section of the three mandibular molars, enlarged 28x. O.P.: occlusal plane, C.A.: crest area of the interradicular septum, F.A.: fundic area.

The photographs at higher magnification were of the regions in which measurements were to be taken.

Measurements The direction of the occlusal plane of the molars was determined from inspection of the survey photograph (Figure 1) and with the aid of suitable landmarks transferred to each of the regional pictures. "Horizontal" measurements of the distance between lead lines and bone surfaces were made parallel and "vertical" measurements at right angles to the occlusal plane. The measured distances were divided by the respective magnifications to obtain the actual amount of hard tissue formation. Daily rates of growth were calculated from the total apposition and the length of the experimental period.

Alveolar bone, interradicular crest (Figure 2) and fundus (Figure 3): Lines parallel to the occlusal plane were drawn at the points of "cresting" of the lead lines and of the alveolar bone margin and "vertical" bone apposition was measured as the vertical distance between the lines. On the operated side of the experimental animals, daily rates for both the first nine and the second seven experimental days were obtained, the former from the distance between the two lead lines and the latter from the distance between the second lead line and the bone surface.

Cementum formation was measured

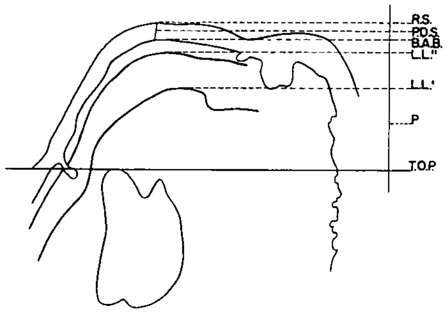

Fig. 2 Enlargement (117x) of the crestal area of the mandibular first molar showing construction of the grid coordinate system and sites of measurement. T.O.P.: transferred occlusal plane, P.: perpendicular to transferred occlusal plane, L.L.': first lead line, L.L.": second lead line, B.A.B.: border of alveolar bone, P.D.S.: periodontal space, R.S.: root surface.

by the same method as bone formation, viz., as the vertical distance between the crest of the lead line and that of the tooth surface (Figure 3).

Posterior surface of the interradicular septum (Figure 1): A representative area, in which the lead lines and bone surface were parallel, was chosen near the middle third of the root. In this area the horizontal distance between the lead line and the margin of the bone was measured.

The width of the periodontal space (PDS) was measured at the interradicular crest (Figure 2) and at the fundus (Figure 3). Measurement of the shortest distance between bone and cementum was made without recourse to a coordinate system. It was felt that this minimized the danger of errors due to tangential sectioning.

Findings

Body Weight

Injection of lead acetate resulted in a drop of body weight. In the control animals the deficit still averaged 21 g five days after the injection, and a considerable number of animals continued to lose weight up to the ninth day. Recovery of the initial weight was not complete at the end of the 16 day period of observation.

In the experimental animals the combined trauma of lead acetate injection and tooth grinding caused a larger initial drop of weight, averaging 47 g on the second day. Recovery, however, was more rapid, and the second lead injection that this group received nine days after the first led to only 16 g average weight loss.

Individual weight losses were found to be unrelated to individual rates of hard tissue formation, except that an experimental animal with exceptionally great loss of weight showed the slowest rate of bone growth.

Alveolar bone formation

All control animals showed a measurable degree of alveolar bone apposition at all investigated sites (Table I). The unoperated (left) side of the ex-

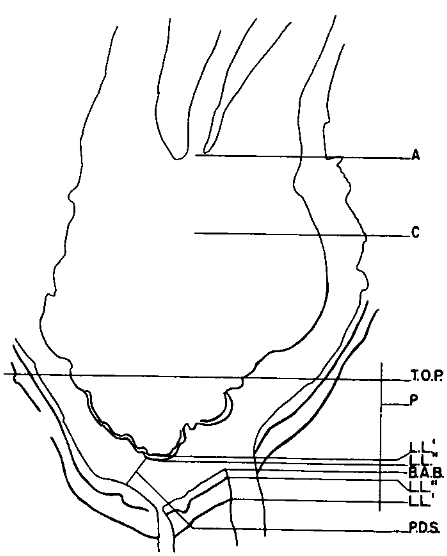

Fig. 3 Enlargement (117x) of the fundic area of the distal root of the mandibular first molar. P.D.S.: periodontal space, L.L.': first lead line, L.L.": second lead line, B.A.B. border of alveolar bone, P.: perpendicular to transferred occlusal plane, T.O.P.: transferred occlusal plane, C.: cementum, A.: apex of root.

TABLE I

Bone Apposition at the Interradicular Septum

(μ/day)

	First Molar		
Controls:	Crest	Fundus	Posterior Surface
Group I - unoperated (left) side	2.14 ± .50	2.50 ± .49	1.54 ± .64
Group II - left side	2.14 ± .87	2.47 ± 1.14	1.17 ± .42
Group II - right side	2.02 ± .75	1.77 ± .79	1.31 ± .41
Average	2.10	2.25	1.34
Experimental:			
Group I - relieved (right) side	12.3 ± 3.51	9.37 ± 3.31	4.69 ± 1.99
Ratio: $\frac{\text{experimental}}{\text{control}}$	5.9	4.2	3.5

	Second Molar		
Controls:	Crest	Fundus	Posterior Surface
Group I - unoperated (left) side	3.60 ± 1.98	3.10 ± 1.14	1.35 ± .36
Group II - left side	2.27 ± .95	2.10 ± 1.81	1.21 ± .19
Group II - right side	1.53 ± .61	1.03 ± .27	1.07 ± .68
Average	2.47	2.08	1.21
Experimental:			
Group I - relieved (right) side	11.6 ± 3.63	8.55 ± 4.12	4.80 ± 1.40
Ratio: $\frac{\text{experimental}}{\text{control}}$	4.7	4.1	4.0

* Figures preceded by ± are the standard errors of the averages.

perimental animals likewise showed bone formation at all sites, usually at rates slightly above those in the unoperated controls.

The three sets of control teeth differed in their average rate of bone formation, though not by statistically significant amounts. The measurements on the left side tended to average more than those on the right, especially for the second molar. These differences were more probably introduced by the asymmetrical approach in sectioning molars from right and left sides of the mandible than by natural asymmetry.

In contrast to findings in young animals where apposition at the crest of the interradicular septum is much more rapid than at the fundus, the present older animals showed at both areas rates of similar magnitude in all three control sets. At the first molar, apposition at the crest was in two sets even slightly smaller than at the fundus, but at the second molar somewhat larger in all three. As in young animals, the rate of apposition at the posterior border of the septum was in almost all cases considerably lower than at the other two sites.

The average of the three sets for the first molar was 2.1 μ/day at the crest, 1.3 μ at the posterior surface of the interradicular septum, and 2.3 μ at the fundus. The corresponding rates for the second molar were similar.

Because the unoperated side of the experimental animals could not safely be considered as representing true control values, the average of the three sets of control measurements was used for evaluation of the experimental effects. Comparison of the experimental daily rates computed on the basis of the first nine and the second seven days of relieved occlusion showed no trend to lower rates in the second period. This indicated that occlusion had not been reestablished. All rates presented below were therefore computed from the whole sixteen day period, that is, from the distances between the first lead line and the hard tissue margins.

There was a marked experimental acceleration of bone apposition at all sites measured. At the first molar, bone apposition on the operated side averaged 12.3 μ/day at the crest, 9.4 at the fundus and 4.7 at the posterior surface of the interradicular septum. The corresponding rates for the second molar were closely similar. These rates represented experimental increases ranging between 3.5 to 5.9 times the control rates. At both molars the acceleration was most marked at the crest of the septum where bone formation was by about 3 μ/day more rapid than at the fundus. The smallest acceleration occurred at the posterior surface of the septum.

Cementum

Only thirteen of forty molars in the control animals and nine of thirty-four molars on the unoperated side of the experimental animals showed measurable apical cementum apposition, i.e., a zone of cementum superficial to the lead line. Most teeth showed only surface deposition of lead along the apical border and three failed to show any deposition. Based on only the teeth with measurable growth, the average daily cementum formation was 1.33 μ in the first, 1.57 in the second molar of the unoperated side of the experimental animals, and slightly less on right and left sides of the control animals.

The behavior of cementum in response to removal of occlusion was in marked contrast to that of alveolar bone. Twenty-six of thirty-four molars with relieved occlusion showed no measurable cementum growth. In eight teeth cementum apposition was measurable, and the rate averaged a similar magnitude as in the controls. The ratios of experimental over control rates were 1.25 for the first molar, and 1.59 for the second. The differences were not of statistical significance.

Periodontal Space

As in the case of the hard tissues, in the control animals there was considerable asymmetry in the measurements of the PDS, more marked at the fundus than at the furcation of the roots. However, the measurements in the three sets of control teeth were of similar magnitude. (Table II) In contrast to young animals, the width at the fundus was only slightly greater than the width at the furcation.

Using the average of the three sets of control teeth as the basis for evaluation for experimental changes, the fundic PDS on the operated side was found slightly enlarged at both molars. The differences amounted to 17 μ in the first molar and 11 μ in the second.

These differences were not statistically significant, but might nevertheless represent a real widening of the fundic PDS in response to relieved occlusion as will be shown in the discussion.

Discussion

The present study doubles the age span investigated by previous workers, and it was thought worthwhile to compile the data in the literature for comparison. Table III shows the dramatic decrease of the physiological growth rates of alveolar bone and cementum in rats of increasing age. In spite of the fact that most studies refer to only one age group and that no two investigators used identical methods, bone formation at the crest and posterior surface of the interradicular septum and at the fundus, and cementum formation at the apex of the first molar show a nearly continuous decrease with increasing age.

TABLE II

Width of Periodontal Space (μ)

	First Molar		Second Molar	
	Crest	Fundus	Crest	Fundus
Controls:				
Group I - unoperated (left) side	90.1	107.3	100.1	91.9
Group II - left side	97.5	120.8	93.3	109.0
Group II - right side	71.3	105.8	85.2	120.0
Average	86.3	111.3	92.9	107.0
Experimental:				
Group I - relieved (right) side	90.5	128.0	100.0	118.1

The older animals of the present study show that the growth rates continue the decline seen in the younger groups, but that growth of bone persists at measurable rates even in animals of quite advanced age.

After the molars have come into occlusion, vertical bone growth serves to keep the teeth in contact. Two physiologic processes tend to produce a space between upper and lower molars: attrition and the downward shift of the mandible due to growth at the condyle. Condylar growth continues after body size and weight have reached a plateau, though at progressively decreased rates.[10] Age changes in rate of attrition seem not to have been studied. It is probable that attrition too slows once body size reaches a plateau, since food intake and mastication then only serve maintenance of muscular activity and basal metabolism.

At the interradicular crest the sole contributor to vertical tooth movement is the apposition of alveolar bone. The growth of cementum makes a negligible contribution, and the width of the PDS does not change with age (Table III). Thus alveolar bone formation alone compensates for attrition and condylar growth. As these slow with age, so does the rate of bone formation. At the end of the rapid growth phase (90-100 days), the rate is about two fifths that in the younger animals. Over the next five to six months it decreases to two fifths of the ninety day value again, and in five more months, it is once more cut in half.

In the fundic region, the rate of hard tissue formation at any given age is the resultant of three processes: formation of fundic bone, formation of apical cementum, and change in width of the PDS.

Growth of cementum declines more steeply with age than growth of alveolar bone. Up to two hundred days of age, the cementum contributes more than half of the total apical hard tissue formation, but in the oldest animals its contribution is less than a fourth. In the animals of the present study only a minority of the roots showed demonstrable cementum formation. The rate at this age represented a reduction to about four per cent of the rate in the youngest animals. Thus growth of fundic bone must take over an increasingly large proportion of apical hard tissue formation.

The need for hard tissue formation in the fundic region is furthermore increased by the decrease in the width of the apical PDS, which narrows from about 240 to about 120 μ between the youngest and oldest age groups. The increasingly greater role of fundic bone formation is reflected in the fact that its rate only decreases to about one fourth of the rate at the youngest age, whereas the rate of interradicular bone formation at the crest decreases to less than one tenth.

In rats, bone formation parallel to

TABLE III

Formation of Alveolar Bone and Cementum and Width of Periodontal Space in Rats of Increasing Age (First mandibular molar, μ/day)

Reference	Strain	No of Animals	Age in Days	Alveolar Bone (Interradicular Septum): Crest	Alveolar Bone (Interradicular Septum): Fundus	Posterior Surface	Apical Cementum	Periodontal Space: Crest	Periodontal Space: Fundus
5	"Albino"*	12	14-21	27.6	7.8				
5	"Albino"*	9	21-35	24.1	7.7				
6	Charles River	10	42-52	26.5	7.8	7.9	15.4	82	236
7	Holtzman	19	51-61	23.3		9.7			
5	"Albino"*	10	49-63	13.5	6.9		12.5		
8	Holtzman	19	54-63	19.7	10.3	6.1	11.5		
6	Charles River	9	56-66	18.4	7.9	9.2	10.3	81	255
2	Sprague-Dawley	4	51-69	19.7		7.0			
2	Sprague-Dawley	5	51-75	21.3		7.9			
2	Sprague-Dawley	4	52-73	18.8		6.5			
7	Holtzman	20	51-71	19.2		8.2		112	241
5	"Albino"*	8	63-77	11.4	6.5		8.7		
5	"Albino"*	9	77-91	10.6	5.9		7.7		
5	"Albino"*	9	91-100	10.1	5.2		6.7		
5	"Albino"*	6	125-200	7.5	3.0		3.0		
9	Sprague-Dawley	8	260-290	3.9	2.3	1.1	2.2	108	104
	Charles River **	29	450-575	2.10	2.25	1.34	.80	83	111

* Unspecified strain of albino rats derived from different sources.
** Present study

the occlusal plane occurs mainly at the posterior surfaces of the bony septa. Growth at the condyle causes a forward as well as a downward displacement of the mandible. Bone formation at the posterior surfaces offsets the forward displacement, and its rate is related to the rate of condylar growth. But in addition, bone formation parallel to the occlusal plane also maintains the mesiodistal dimensions of the alveolar septa during tooth eruption at a rate related to the rate of eruption.[8] The rates of bone formation at posterior surfaces thus reflect the age changes both in rate of condylar growth and of eruption. They are furthermore complicated by changes in the shape of the molar roots due to irregular apposition of cementum, and it is difficult to obtain reliable measurements of posterior bone apposition. The rates reported by the different investigators show greater discrepancies than those at the other sites. They suggest a reduction to about one seventh between the youngest and oldest animals.

Since the mandibles as well as the molars are structures of limited size, the growth at the condyles and of apical cementum must slow down when their size limit is approached. The rate of alveolar bone growth, on the other hand, is at any age which is necessary for keeping the molars in occlusion, and higher rates than those necessary are prevented by masticatory forces.[2] The striking progressive reduction with age in the rates of alveolar bone formation thus may reflect no more than the reduced rate of condylar growth, leading to reduced rates required to keep the masticatory apparatus adjusted to the changing dimensions of the mandible. The observed striking progressive reduction with age of condylar growth, apical cementum and alveolar bone formation (Table III) therefore does not reflect slowing down of bone growth due to old age in the sense usually implied by this phrase.

The purpose of the present study was to investigate the growth potential of the molar-alveolar unit in rats of advanced age, as revealed during release of occlusion. Bone growth in response to relieved occlusion was tested at the same sites at which a striking decrease in the physiologic rates of bone formation had taken place, i.e., interradicular crest, fundic bone, posterior surface of the interradicular septum, and apical cementum.

It was found that the advanced age of the animals did not prevent the alveolar bone from responding with an acceleration amounting to a multiple of the rates of growth in control animals of this age. Expressed in these terms, the response of the old animals was nearly twice as great as the response of the young animals studied by Schneider and Meyer (Table IV): a five to sixfold increase occurred at the crest of the interradicular septum and a fourfold increase at the fundus. The increase at the posterior surface of the interradicular septum also was slightly higher than in the younger animals, but bone apposition at this site cannot be measured reliably enough to permit meaningful quantitative comparisons.

The daily amount of bone formed in the old animals was a fourth to a third of that in Schneider and Meyer's young animals. Nevertheless, the animals were able to return to the rates physiologically seen in growing young animals at two months of age or less. And if growth potential is measured as the capacity for acceleration over the pre-experimental rates of growth, then the old animals manifest an even greater potential for alveolar bone growth than do young animals.

The apical cementum responded only weakly to the relief of occlusion, whether viewed with respect to the

TABLE IV

Hard Tissue Formation in Response to Relieved Occlusion in Young and Old Rats (First Molar)

Reference	Age	Alveolar Bone at Interradicular Septum						Cementum	
		Crest		Fundus		Posterior Surface		Apex	
		µ/day	Acceleration*	µ/day	Acceleration*	µ/day	Acceleration*	µ/day	Acceleration*
2	54- 63	47.5	2.4	26.7	2.6	18.09	3.0	14.80	1.3
Present Study	450-560	12.3	5.9	9.4	4.2	4.69	3.5	1.03	1.4

* Ratio of experimental and control rates

number of animals stimulated into resuming growth of cementum, the number showing accelerated growth, or the degree of acceleration in those that did. However, cemental response was of the same slight degree in Schneider and Meyer's young animals as in the present old animals. The reason why cementum fails to respond to relieved occlusion might be that this represents an inadequate stimulus or, alternatively, that cementum is not able to respond to experimental stimuli. The marked response of apical cementum to growth hormone observed by Meyer, Schneider and Das[9] in animals of intermediary age is evidence against the second of these alternatives.

The failure of cementum to respond to relieved occlusion places nearly the entire burden of excess hard tissue formation in the fundic region of the experimental animals on growth of fundic bone. A comparison was made of hard tissue formation at the interradicular crest and at the fundus. The highest possible estimate of the contribution of cementum was used for the fundic region by including as cementum formation the width of the apical lead band on those teeth that showed no cementum apical to the lead line. Even using this estimate, a daily deficit at the fundus of about 2 μ per day was noted both at the first and the second molars, which during the sixteen day experimental period adds up to a deficit of 32 μ. This negative balance of hard tissue formation at the fundus compared with the crest makes it likely that the widening of the PDS, even though statistically dubious, actually took place. This is all the more likely since a similar degree of widening of the apical PDS was noted in Schneider and Meyer's young rats as well. It appears that at both ages the fundic bone could not quite keep pace with the accelerated eruption of the teeth resulting from the accelerated bone formation at the interradicular crest. This may be the limiting factor in the response of molars to relieved occlusion.

Summary

The effect of release of molars from occlusion on alveolar bone growth was studied in aged rats. Twenty male albino rats of the Charles River strain 450 days of age and ten 560 days of age were given an intravenous injection of lead acetate as a vital marker for hard tissue formation. In the twenty younger animals, the right maxillary molars were ground to the gingival level to release the right mandibular molars from masticatory function. The contralateral side and the unoperated animals were used as controls. Hard tissue formation was measured on photomicrographs of histological sections of the mandibular molars. Rates of alveolar bone growth were measured at the crest, fundus, and posterior surface of the interradicular septum. Cemental growth was measured at the apices of the distal roots of first and second molars.

All control animals showed growth of alveolar bone at these sites at rates between ten and twenty-five per cent of the physiologic rates at about fifty-five days of age.

Experimental relief of occlusion caused accelerated alveolar bone growth at the sites of physiological growth. Although the amount of bone formed was less than in young animals, the degree of acceleration was markedly higher in the aged rats, revealing a growth potential comparable to physiologic rates of alveolar bone formation seen in animals at sixty days of age. Apical cementum formation had ceased in most controls. In agreement with findings in young animals, relief of occlusion caused only a feeble response of cementum in the experimental animals.

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