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The Rate of Osteoclastic Bone Erosion in Haversian Remodeling Sites of Adult Dog's Rib*

Z. F. Jaworski and Eric Lok

Department of Medicine, Ottawa General Hospital and University of Ottawa, Ontario

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Using tetracycline labelling *in vivo* and histological analysis of undecalcified longitudinal sections of cortical bone, the average speed was measured with which osteoclastic fronts (cutting cones) of the evolving Haversian systems erode bone. This speed reflects the function of the individual osteoclasts within the cutting cone and is opposite to the appositional rate (the thickness of bone deposited by a monolayer of osteoblasts in unit time). In fifty-three Haversian remodeling sites in several sections from four different ribs of three adult dogs, the longitudinal erosion rate (in the direction of long axis of the osteon) averaged 39 ± 14 μ/day, whereas the radial erosion rate, that is the centrifugal enlargement from the tip of the cutting cone to its largest diameter, averaged 7.1 ± 3 μ/day.

Since human and canine lamellar bone remodeling dynamics appear closely similar, the estimates in this study may also apply to man.

Key words: Osteoclast — Bone — Erosion — Haversian system — Dog.

Par marquage *in vivo* à la tétracycline et analyse histologique de coupes non décalcifiées d'os cortical, la vitesse moyenne d'érosion des fronts ostéoclastiques (cones coupants) de systèmes de Havers a été mesurée. Cette vitesse traduit la fonction d'ostéoclastes isolés dans le cône coupant et est l'inverse de la vitesse d'apposition (l'épaisseur de l'os déposé par une couche unique d'ostéoblastes par unité de temps). Au niveau de cinquante trois zones de remaniements haversiens, dans plusieurs coupes de quatre côtes différentes de trois chiens adultes, la vitesse longitudinale d'érosion (dans la direction de l'axe longitudinal de l'ostéone) est d'environ 39 ± 14 μ/jour, alors que la vitesse d'érosion radiaire, c'est-á-dire l'élargissement centrifuge du sommet du cône coupant vers son plus large diamètre, est d'environ 7.1 ± 3 μ/jour. Etant donné que la dynamique du remaniement osseux lamellaire de l'homme et du chien est très semblable, ces résultats peuvent aussi être appliqués á l'homme.

Es wurden *in vivo* Tetracylin-Markierung und histologische Analyse von unentkalkten Corticalis-Längsschnitten verwendet, um die durchschnittliche Geschwindigkeit zu messen, mit der die Osteoclastenkegel (cutting cones) der sich entwickelten Havers'schen Systeme im Knochen vorrücken. Diese Geschwindigkeit reflektiert die Resorptionstätigkeit eines einzelnen Osteoclasten, im Gegensatz zu Appositionsrate, welche die Intensität der Matrixproduktion durch die Osteoblasten in einer bestimmten Zeitspanne angibt. Bei 53 im Umbau begriffenen Havers'schen Systemen in mehreren Schnitten von vier verschiedenen Rippen dreier erwachsener Hunde war die durchschnittliche Geschwindigkeit des longitudinalen Vorrückens 39 ± 14 μ/Tag, während die durchschnittliche Erweiterung des Durchmessers an der Spitze des Resorptionskanales einen Betrag von 7.1 ± 3 μ/Tag ausmachte.

Da die Dynamik der Umbauvorgänge im kompakten Lamellenknochen bei Hund und Mensch vergleichbar ist, kann angenommen werden, daß die ermittelten Werte auch für den Menschen zutreffend sind.

For reprints: Z. F. Jaworski, M.D., F.R.C.P. (C), Department of Medicine, Ottawa General Hospital, 43 Bruyére Street, Ottawa 2, Ontario, Canada.

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Introduction

The average thickness of new lamellar bone deposited *in vivo* by a monolayer of osteoblasts in unit time (the appositional rate) has been measured directly and accurately in several species of mammals, including man, by the use of *in vivo* markers, such as tetracycline [5]. In contradistinction, the speed with which osteoclastic fronts erode[1] bone *in vivo* cannot be measured *directly* by histological means. Indirect estimates of that speed vary from 3 μ to 300 μ per day, according to the method used [4, 10]. Although some clues as to bone resorption and formation activity can be derived from measuring the length of the resorption and formation surface perimeters in cross-sections of compact or spongy bone [11, 13, 15], from such static parameters one cannot reliably infer the amount of bone eroded or formed [5, 6, 17].

Since the appositional and bone erosion rates depend respectively on individual osteoblast and osteoclast activity [4, 5], various factors which in health and in disease affect the vigor of these cells could also alter transiently or permanently the bone tissue turnover rate and balance on the one hand [9, 17, 18] and the histologic appearance of bone on the other. A more accurate measurement of the osteoclastic bone erosion rate therefore has theoretical and practical value.

We describe here a method of making that determination based on tetracycline labelling and on histological analysis of undecalcified longitudinal sections of cortical bone.

Material and Methods

Material, Biopsies and Labels

Three one-year old male dogs (two from the same litter) weighing approximately 20 kg were given two intramuscular injections of tetracycline, 20 mg/kg, with a 7 day interval between injections. Biopsy of the middle $^1/_3$ of the 11th rib was taken from 1 to 11 days after the last tetracycline label. One dog (No. 3) underwent biopsy 1 day and again 8 days after the last label; whereas the two remaining dogs were biopsied respectively on the 7th and 11th days after the last label.

Throughout the experiment, the dogs were kept unrestrained in the animal quarters and were fed Purina dog chow and water *ad lib.*

Sections

Longitudinal sections of the cortical bone were hand-ground under running water to a thickness of 70 μ, stained and mounted as described by Frost and Villaneuva (2, 16). The sections were inspected under bright and fluorescent light (the latter for tetracycline identification) under 160 × magnification to measure newly-developing Haversian systems.

Rationale

Because Haversian systems are aligned parallel to the long axis of bone, thick longitudinal sections of the cortex may contain several actively forming Haversian systems. In such systems one can distinguish the *cutting cone* containing osteoclasts, and behind, the reforming osteon or *closing cone* (10) characterized by the bands of unmineralized osteoid on both sides of the central canal (Figs. 1 and 2).

1 The term "bone erosion" rather than "resorption" will be used in this text to designate the morphological evidence (such as Howship's lacunae) of bone removed by osteoclasts. The term "bone resorption" is often used in the literature to denote the net flux of mineral from bone into the surrounding fluid (*in vivo* or *in vitro*) resulting from a variety of mechanisms which may, or may not, be associated with morphologically observable loss of bone substance.

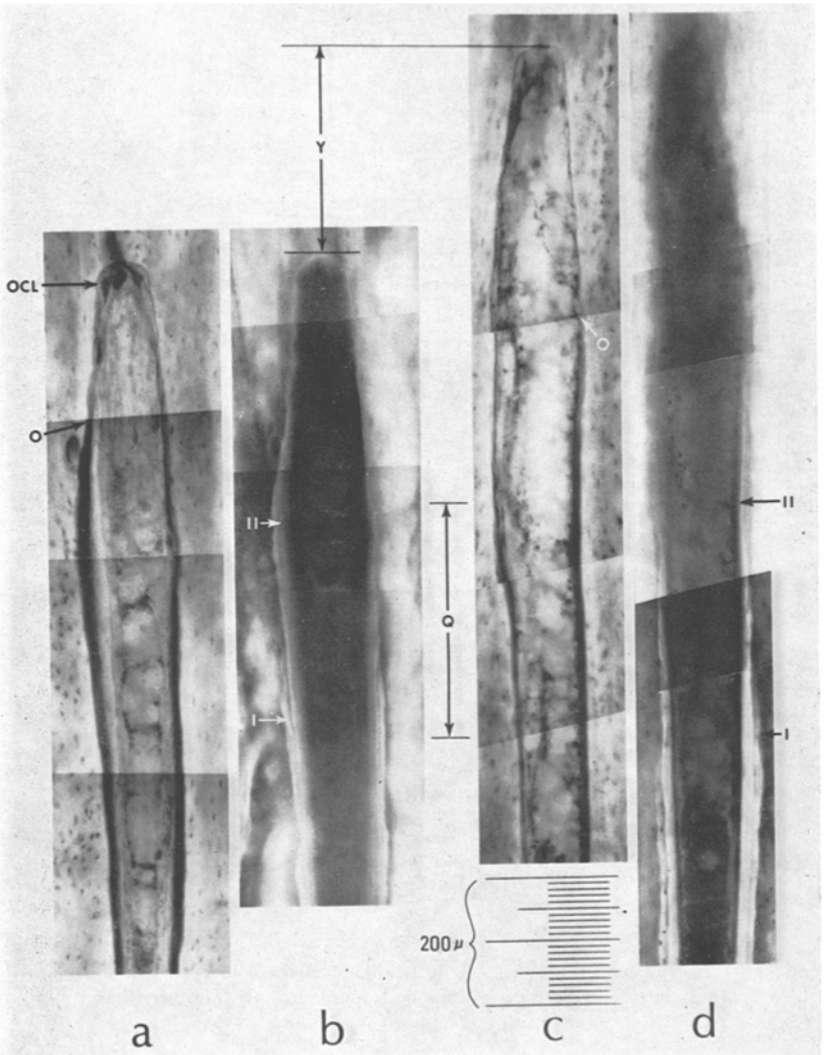

Fig. 1. Photomicrograph (*a* and *c* under bright light and *b* and *d* under fluorescent light) of two typical haversian systems one day (left panel) and eight days after the last of two tetracycline labels (right panel). *OCL* Osteoclasts; *O* Non-label osteoid; *I* and *II*, the first and second labels respectively; *Q* segment between proximal ends of two labels. The segment *Y* represents the distance which the population of evolving labeled osteons advanced through the bone between the first and eight day after the last label

Tetracycline, is deposited with the mineral at the calcification front where it appears in undecalcified bone sections as a yellow band under blue fluorescent light. In *cross-sections* two separate injections of tetracycline given at known intervalls will appear in all actively forming osteons as two fluorescent concentric rings [5]. In *longitudinal sections*, on the other hand, they will appear as two longitudinal parallel bands, lying on each side of the Haversian canal of an actively forming Haversian system as illustrated in Figs. 1 and 2; furthermore, the most recent of the two markers will extend closer to the base of the cutting cone than the older one located

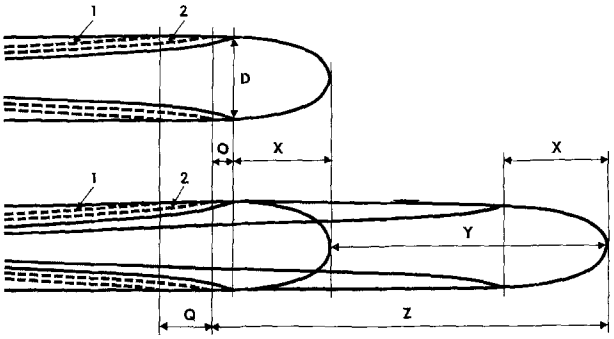

Fig. 2. Schematic representation of a longitudinally transected active haversian system from a biopsy taken on the day of the last tetracycline label (above) and one taken several days after (below). The segment *Y* between the tips of the cutting cone above and one below is the distance which the erosion front traversed during the time between the biopsies. For a detailed explanation, see the text

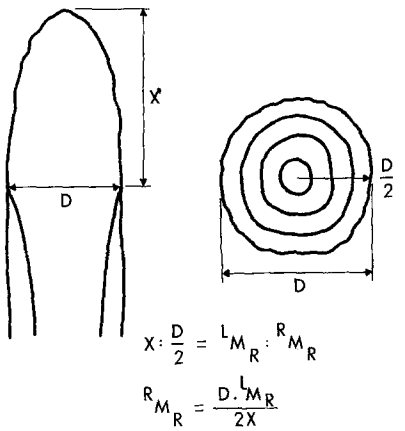

Fig. 3. Schematic representation of the longitudinal (on the left) and cross-section (on the right) of a cutting cone, illustrating the rationale (explained in the text) for calculating the radial bone resorption rate

externally to it because during the time interval between the two labels, and extending or trailling behind the advancing cutting cone, new layers of osteoid deposit over the initial marker. If, labelled bone is taken at intervals from the mid-third of two opposite ribs of the same subject, in the longitudinal section the proximal end of the *last label* will mark the site where at the day of labelling eroding osteons began to mineralize. Because of the resorption taking place after the last label, the tips of the cutting cones will come to lie further and further ahead of the forward edge of either label (Figs. 1 and 2).

In order to calculate the rate of advance of the cutting cone per day, the distance *Y* must be obtained. This is the elongation of the cone occurring between the last labelling and the biopsy. Enough adequately transected, labelled, evolving osteons must be found in the longitudinal setions to measure the *mean* distance between the tips of the cutting cones and the proximal ends of the last label (distance Z in Fig. 2). Such a measurement may be based on one biopsy taken after the last label, or two or more successive biopsies after the last label from equivalent sites as described in the following sections.

The estimate of the *radial erosion rate*, that is the speed with which the cutting cone enlarges from its tip to the maximal diameter at its base as seen in a cross-section, is based on the following reasoning:

It takes exactly the same time for the cutting cone to advance its whole length and to expand laterally from the tip to its largest diameter (radial erosion). Consequently, the ratio of the longitudinal to radial erosion rate must equal the ratio of the length of cutting cone (segment X) to its radius ($D/_2$) at the base (planing cone), as depicted in Fig. 3.

Measurements

At 160× magnification (using a calibrated micrometer eyepiece), both the *lengths* of cutting cones from their tips to the point where osteoid first appears [2] (segment X in Fig. 2), and their largest *transverse diameters* (segment D) were measured. Then, under fluorescent light the lengths of the segment Z (from tip of the cutting cone and the proximal end of the last label) and segment Q (distance between the proximal ends of the two labels) were measured. The mean values for all systems measured appear in Table 1.

Calculations

A. The Mean Longitudinal Erosion Rate ($^{L}M_{R}$):

Method 1: In Dog No. 3 biopsied 1 day and 8 days after the last label, the difference between the mean distance of segment Z in the first and second biopsies divided by the interval between the biopsies in days equals the mean longitudinal erosion rate as shown in Fig. 2, according to the equation:

$$^{L}M_{R} = \frac{Z_2 - Z_1}{t} \tag{1}$$

where Z equals the distance from the tip of the cutting cone to the proximal end of the last label and t equals the interval between the two biopsies in days.

Method 2: Given only one biopsy taken after the last label, $^{L}M_{R}$ is obtained from the equation:

$$^{L}M_{R} = \frac{O}{t+1} \tag{2}$$

where t equals time interval between the last labelling and biopsy in days and O equals the length of the non-labelled osteoid; since O is not measured directly, it is obtained from the equation:

$$O = Z - X. \tag{3}$$

B. The Mean Radial Erosion Rate ($^{R}M_{R}$):

The rate of radial erosion, that is the speed with which the cutting cone enlarges from its tip to the maximal diameter at its base (segment D in Figs. 2 and 3), is calculated from the equation explained above and in Fig. 3.

$$^{R}M_{R} = \frac{D \times {}^{L}M_{R}}{2X} \tag{4}$$

where D equals the largest diameter of the cutting cone and X equals its length. Both are known from direct measurements, whereas $^{L}M_{R}$ is obtained from the Equs. (1) or (2).

C. The longitudinal Bone Formation Rate ($^{L}M_{F}$ or q) is obtained from the aquation:

$$q = \frac{Q}{t} \tag{5}$$

where Q equals the *mean* length of the segment between the proximal ends of both labels and t equals the interval in days between the two labels (seven days in each instance).

2 Because the ends of various segments on both sides of the central canal are usually asymmetrically located, an intermediate position between the two is taken to measure the cutting point of the segment.

Table 1. Osteonal measurements and rates

Subject	Last label-biopsy interval (days)	Number of osteons	X[a] (μ)	D[b] (μ)	Z[c] (μ)	Q[aa] (μ)	O[bb] (μ)	${}^{L}M_{R}$[cc] (μ/day)	${}^{R}M_{R}$[ab] (μ/day)	q[ac] (μ/days)
Dog No. 3	1	8	350 ±71	129±35	393±74	282 ±88	43.0±16			40.3±13
Dog No. 1	7	14	385 ±112	127±18	681±198	351 ±107	296±99	37.0±12	6.2±1.5	50.2±15
Dog No. 3	8	16	308 ±98	123±27	650±176	312 ±61	342±99	37.3±11.3	7.8±2.9	44.5±9
Dog No. 2	11	14	336 ±96	105±29	853±222	285 ±69	521±205	44.4±17.1	7.2±3.7	40.7±9.5
Mean±SD			342.6±100	120±28		310.5±84		39.2±13.7	7.1±2.9	44.4±12

[a] X = length of the cutting cone from the tip to its largest diameter.
[b] D = largest diameter of the cutting cone at its base.
[c] Z = distance from the tip of the cutting cone of the proximal end of the last label.
[aa] Q = distance between the proximal ends to the first and the second (last) label.
[bb] O = length of the non-labeled osteoid.
[cc] ${}^{L}M_{R}$ = longitudinal erosion rate.
[ab] ${}^{R}M_{R}$ = radial erosion rate.
[ac] q = longitudinal mineralization and osteoid deposition rate.

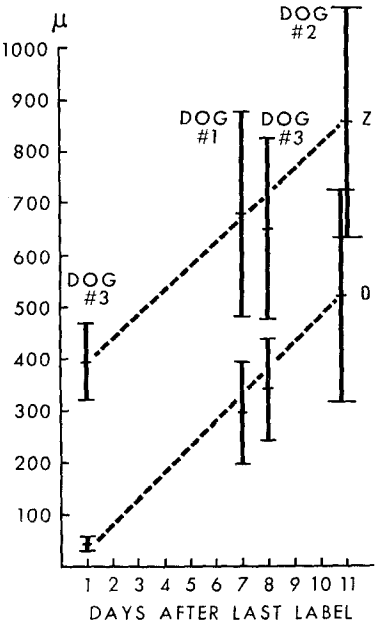

Fig. 4. The plot of the mean distance between the tip of the cutting cones and the proximal ends of the last label (segment *Z*) and the lengths of the non-labeled osteoid (segment *O*) measured in sections from the rib, biopsied, one, seven, eight and eleven days after the last label

Results

Table 1 presents the measurements and rates obtained in the present study.

The mean length of the cutting cone obtained in fifty-two active osteons was $343 \pm 100\ \mu$ and the mean largest diameter at the base was $120 \pm 28\ \mu$. As compared with the dimensions of cutting cones in the human rib, 250–400 μ and 187.5-250 μ [7, 10] respectively, they appear of approximately the same length, but are considerably narrower.

As shown in Table 1, the values for the *longitudinal erosion rate* obtained by a single biopsy method (Method 2) in three dogs, each biopsied seven, eight and eleven days after the last label, ranged from 37—44.4 μ/day, with the mean for the three dogs (a total of 44 osteons) of 39.2 μ/day.

In dog No. 3 biopsied one day and eight days after the last label, we calculated the longitudinal and radial erosion rates by both methods described with similar results; using the two-biopsy approach (Method 1), the ${}^{L}M_{R}$ averaged 36.7 μ/day, whereas the single biopsy method (Method 2) gave 37.3 μ/day.

Fig. 4 shows the mean distance between the tips of the cutting cones and the proximal end of the last label (segment *Z*), as well as the mean lengths of the unlabelled osteoid (segment *O*) in the biopsies taken on days one, seven, eight and eleven after the last label. The values fall on a straight line showing that during the eleven-day period of observation, the cutting cones advanced through the bone and new osteoid deposition on the wall of eroded cavities proceeded at the same and essentially constant mean rate of approximately 44 μ/day.

The length of segment Q (between the proximal ends of the first and second labels) averaged 310 ± 84 μ for 52 osteons in four biopsies measured. Since seven days separated the administration of two labels, the *longitudinal* osteoblastic bone deposition and mineralization (value q) averaged 44.4 ± 12 μ/day value similar to that of longitudinal resorption rate calculated from separate measurements. This proves both the constant morphological relationship between various segments of evolving Haversian systems and the reliability of estimating the rate of osteoclastic erosion rate by this method.

The radial erosion rate within the Haversian remodeling sites ranges in three dogs from 6.2–7.8 μ/day with a mean for three biopsies and 44 osteons of 7.1 ± 2.9 μ/day.

Discussion

We found that cutting cones of secondary remodeling Haversian systems advance through the cortex of the rib of the adult dog at approximately 40 μ/day. The radial expansion (from the tip of the cutting cone to its largest diameter at the base) averaged 7 μ/day. The described method of measurement is the most direct of the histological methods so far used to evaluate these functions and gave a reproducible result.

We do not know to what extent the rate of osteoclastic bone erosion found in Haversian remodeling systems applies to other osteoclastic erosion sites in the same subject, or how it varies with age and in various species. Johnson estimated the longitudinal erosion rate at 100 μ/day [10]. Sandison [14] and Kirby-Smith [12], observing microscopically the miniature fractures of bone in a transparent chamber placed in the ear of a rabbit, estimated the rate of osteoclastic advance at 60 μ/day, a value close to that found here. Since the configuration, dimensions and dynamics of human and canine lamellar bone remodeling show great similarity, the values for osteoclastic erosion rates observed in this study in the dog may apply to the Haversian system of man as well.

Frost found the radial erosion rate to approximate 3 μ/day, both in man [4] and in the dog [9]. It is somewhat lower but of the same order of magnitude as the 7 μ/day found in our study. Frost's rates of radial erosion depend on measuring in the cross-sections of the cortex the length of the resorption surface perimeter, containing recently described, small resorption cavities. Since these cavities are functionally distinct from Haversian cutting cones [7], the agreement between his values and ours may be even closer.

The difference between the longitudinal and radial rates of erosion (despite equal functional competence of the cells) arises from the geometry of remodeling (or any osteoclastic erosion) sites. The factors which determine the configuration and dimensions of remodeling sites are not understood. For example, in contrast to the tubular shape of Haversian erosion processes within cortical bone, such sites on endosteal surfaces have a broader base and the erosions are larger and flatter [1, 8]. Possibly the direction of erosion relates to the elaborate movement of osteoclasts on the erosion fronts [10] so that, although the erosion front moves in the direction of the main thrust with the same speed, the individual osteoclasts may move more sideways than forward. The radial erosion rate, on the other hand, appears to be the result of geometry and configuration particular to the

Haversian cutting cone which enlarges centrifugally as it advances. The shape of an erosion front in bone sections depends on the plane of sectioning. Since the osteons are aligned parallel to the long axis of the bone, the effect of both longitudinal and radial (centrifugal) bone erosion can be seen on the longitudinal sections of the cortex, but only the radial erosion will be observed on the cross-section (Fig. 4).

The osteoclastic erosion rate, like the appositional rate, is a property of cellular units of bone turnover (or basal metabolic units of Frost) which are found *in vivo* but cannot yet be studied in tissue culture or *in vitro* [3]. The rate relates only indirectly to the activity of the individual osteoclast, that is to the amount of bone removed per cell (or, more precisely, per cell nucleus) in unit time. The reasons are: 1. the advance of osteoclasts through bone occurs as successive waves of cells, the life-span of which may approximate 24–28 h [10]; and 2. because of the elaborate movements of the osteoclast, only a fraction of its work contributes to the actual advance of the front. To the extent, however, that this contribution remains proportional to the total performance of the cell, the advance rate of the erosion front may provide an index of osteoclastic function, and can be evaluated by the method we describe.

From the point of view of the bone tissue turnover *in vivo*, the osteoclastic erosion rate, like the bone appositional rate, constitutes a basic parameter which, along with the measurement of total resorption surface perimeter per unit bone surface area can serve to evaluate the rate of bone tissue resorption. Hence a means of directly measuring the osteoclastic erosion rate may help to identify more precisely the contribution of osteoclastic function to the alteration of bone structure in health and disease.

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