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Haversian and Endosteal Bone Formation Rates in Rib Biopsies of 50 Patients with Senile and Postmenopausal Osteoporosis

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Haversian and Endosteal Bone Formation Rates in Rib Biopsies of 50 Patients with Senile and Postmenopausal Osteoporosis

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Eleventh rib biopsies of 50 patients with senile and postmenopausal osteoporosis, studied by means of tetracycline bone labeling, revealed significant and similar although modest decreases in bone formation on the cortical-endosteal and haversian surfaces of the biopsies.

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THE term, osteoporosis, signifies a disease characterized by the presence of too little bone in the skeleton,[1,2] and it can occur in association with several endocrine disorders,[3-7] certain gastrointestinal tract disturbances,[8] dietary deficiencies,[9] physical and chemical agents, hematological diseases, congenital disorders and in senile involution,[10] as well as in association with other factors and affections.[6, 11, 12, 13] Our discussion will be confined to the senile and postmenopausal forms (SO and PMO), which probably occur most commonly,[13, 14] yet lack effective ways for prevention or treatment.

Albright[4,5] originally proposed that a deficiency of estrogen in women and of androgen in men caused SO and PMO. Later, Reifenstein[7] postulated that a relative increase in the ratio of adrenal glucocorticoids to the sex hormones might be the cause. He advocated corrective and anabolic steroid therapy. Among others, Nicolayson, Eeg-Larsen and Malm,[15] and Nordin,[2, 3] have suggested as causes a chronic calcium deficiency brought about by inadequate diet or by poor absorption in the G.I. tract and/or excessive elimination of calcium.

However, Urist[16] among others showed that blood concentration and urine excretion of estrogen, 17-ketosteroids, 17-hydroxycorticorticoids

and their derivatives did not differ significantly between patients suffering from osteoporosis and normal persons of comparable age. In addition, the great majority of patients with SO and PMO have consistently normal serum and urinary calcium and phosphate value. Also, Smith and Frame found that analyses of dietary calcium intake in victims of SO and PMO did not support the postulated dietary insufficiency.[15] Finally, while trials of anabolic hormones and supplemental dietary calcium and phosphate have yielded conflicting results in experimental situations, they have uniformly failed to cure these diseases in clinical practice. Further studies of the skeletal features of SO and PMO might yield new clues as to their underlying causes, we believe.

An osteoporotic skeleton might present: (1) an increased intracortical porosity, and/or (2) excessively thinned cortex relative to the outside diameter of the bone. Numerous observers have shown by a variety of methods that excessive thinning does characterize the osteoporotic cortex,[17] and one quantitative study of internal cortical porosity revealed that normal intracortical porosities accompanied the thin cortices and (probably) expanded marrow cavities in ribs from humans with SO and PMO.[18]

Frost points out that the *relationship* between bone formation and bone resorption constitutes the key factor, because osteoporosis can occur with increased, normal, or decreased bone formation; or resorption, as long as resorption significantly exceeds formation.[20] If at the time of skeletal maturity, there is a normal skeleton, the subsequent development of an osteoporosis would prove that excess resorption had arisen. The next question is what constitutes the mechanism of this excess?

Within the past decade, morphometry has revealed in ribs[20-22] and in many other bones,[10, 17, 23, 24] that bone contains three functionally as well as anatomically distinct surfaces: *the endosteal, haversian,* and *periosteal,* conveniently termed "envelopes." Each of these envelopes can behave uniquely in health as well as in disease. For example, endosteal bone surfaces normally and almost always have a net loss, while periosteal bone surfaces simultaneously normally have a net gain. Yet, the haversian envelope normally undergoes no major gain or loss. The normal pattern of resorption-to-formation ratios on these envelopes causes a gradual enlargement both of the marrow cavity and of the outside diameter of a bone throughout life. Deviation from the normal pattern, either of degree or in kind, will cause a pathological skeletal state.

These facts suggest that, in theory, envelope-specific disease could develop, a possibility verified by a number of human and animal studies.[18,25,26] Available evidence indicates that SO and PMO represent an excessive bone loss *which occurs primarily on the endosteal envelope.*[18]

If this is so, it becomes important to know how rapidly resorption and formation proceed on each of the three bone envelopes in SO and PMO.

By means of quantitative histological measurements based upon tetracycline bone labeling,[12, 26-33] our study reinforces the findings of an earlier study of bone formation based on a limited number of cases.

Materials

Fifty patients (13 males and 37 females), all with clinical and radiological evidence of osteoporosis, were included in this study. All had back pain attributed to their osteoporosis, thirty of them had one or more compression fractures of the

spine which either occurred spontaneously or followed trivial trauma or physiologic mechanical stresses to the spine. Seven others had experienced rib or metatarsal fractures following trivial trauma. All the fractures healed normally. Their age range was from 31 to 74, with a mean of 55.9 years. Patients with evidence of any other known form of metabolic bone disease were excluded from this study. Serum calcium and inorganic phosphate determinations, obtained on multiple occasions, revealed normal values. Twenty-four-hour urinary calcium excretion determinations, done in 38 of these patients, revealed eight with normal values (ie, 75mg/day). However, these eight people had no other evidence of osteomalacia. As in the other 42 cases, bone biopsies revealed no osteomalacia.

Methods

The analytical and methodological procedures employed constitute those of Frost.[19,20]

1) Labeling, Biopsy and Sections: Oral Declomycin was given as a bone marker, 300 mg t.i.d. orally on a schedule of 3 days on, 10 days off, and 6 days on (3-10-6). This schedule provides an unlabelled interval, and the bone mineral deposited during that interval was the major fraction measured. Continuous labels were avoided, in part because Hong et al[34] and Saxen[35] have shown that tetracyclines depress bone formation. This introduces an uncorrected measurement error of unknown magnitude.

Between one and three weeks after completion of labeling, approximately 3 cm of the 11th rib was removed at the junction of its middle and distal thirds, usually under local anesthesia.[36] At least three fresh, mineralized, complete, and accurately oriented cross-sections; 50-70 microns thick, were made of each rib biopsy by hand grinding under running water on waterproof sandpaper.[37] These were stained with the Villanueva Tetrachrome bone stain,[38,39] dehydrated in ascending strengths of alcohols, cleared in xylol and then mounted for permanent reference in Harleco Synthetic Resin microscopic mounting medium.[17,18]

2) Measurements: Areas and perimeters were measured with a rapid and accurate grid method.[26,29,31,32,40-42] The following measurements were made:

a) Mean cortical cross section area per section (A_C): Representing the cross sectional area enclosed between the periosteal and the endosteal perimeters of the sections, it was measured on each section in mm^2 to an accuracy of one part in 20 and a precision of one part in 30.

b) Endosteal perimeter per section (^eS): Signifying the cortical endosteal boundary or perimeter of the marrow cavity space, it was measured in each section in mm to an accuracy of one part in 20 and precision of one part in 30.[18,43]

c) Mean perimeter of individual haversian osteoid seams (^hS_F), and cortical endosteal osteoid seams (^eS_f): These equal the sum of all the individual haversian or endosteal osteoid seam perimeters in all the sections of a case (measured with the Zeiss integrating eyepiece II[26,49] at 320X, to an accuracy of one part in 20 and precision of one part in 30), divided by the total respective number of seams (^hA, ^eA), the latter counted in bright field microscopy at 128X to an accuracy of one part in 40 and precision of one part in 60.

d) The total number of osteoid seams (^hA and ^eA) as well as of tetracycline-labeled osteoid seams were counted in each section, the former in brightfield microscopy at 128X, the latter under

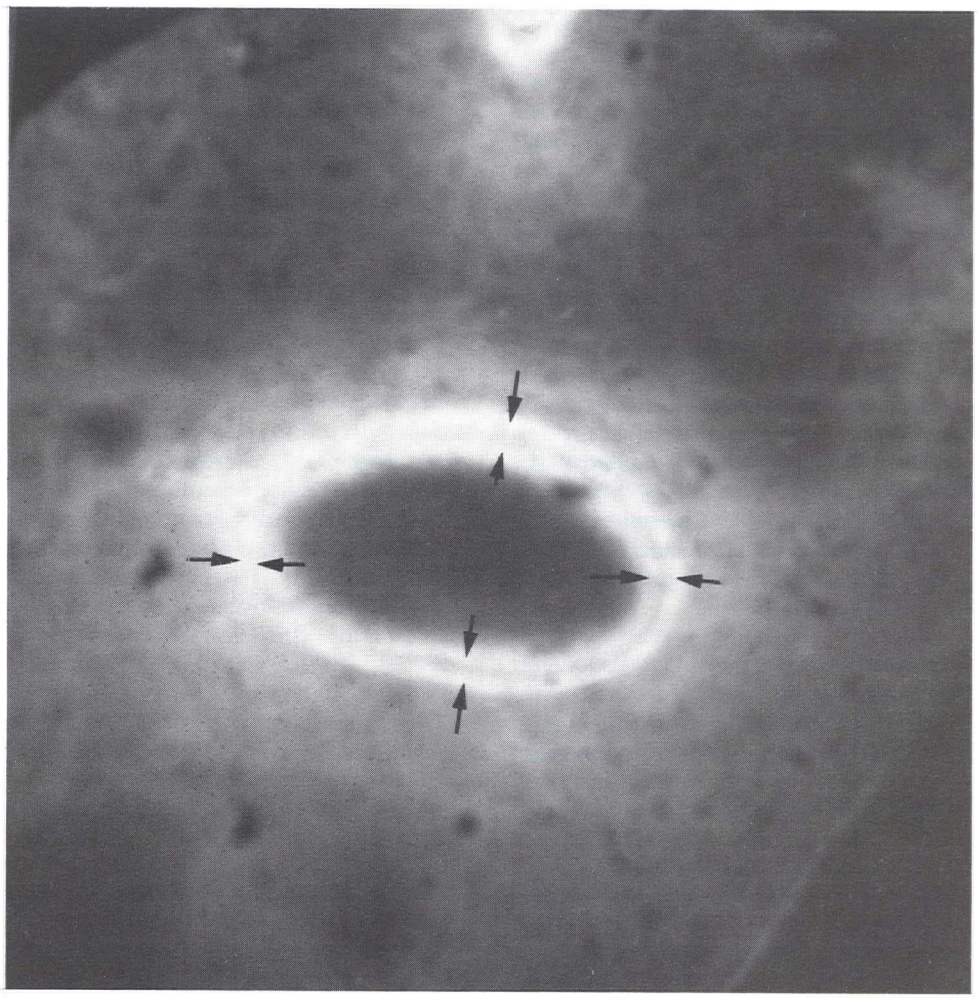

Figure 1
Undecalcified cross section of a rib biopsy at 240X and under fluorescence microscopy, showing two annular bright rings which represent tetracycline labels separated by a 10-day label-free interval. The arrows show where four separate measurements of the distances between the middles of the two bands might be made; their mean divided by the labelling interval (here 13.0 days) would equal the appositional rate for this one haversian system. All such systems in the sections of any given case would be measured similarly to obtain the mean value for the case.

bluelight fluorescence microscopy, at 128X, with a Zeiss fluorescence photomicroscope. The decimal fraction of seams that "took" the labels equalled the total labeled seams divided by the total number of seams labeled plus unlabelled seams, separately for the haversian and endosteal envelopes. The means of all sections per case were listed for later calculations.

e) Tetracycline inter-band distance: Defined as the average distance between the middle point of each of two temporally adjacent tetracycline bands, it was measured with a calibrated eyepiece mi-

crometer under bluelight fluorescence at 320X, at five equally spaced intervals around the perimeter of each labeled system, as shown in Figure 1. Accuracy in the worst-case equals one part in 30.[44] The average of all of these values per case was computed and listed for subsequent calculations, separately for haversian and cortical-endosteal surfaces.

Table I lists the above data, as well as age-comparable group normals.

3) Computations: The following derived parameters were calculated from the above data:

a) Haversian osteoid seam distribution ($^{h}A_f$), per mm² of compacta, and endosteal osteoid seam distribution ($^{e}A_f$) per mm of endosteal perimeter. In symbols:

$$^{h}A_f = \frac{^{h}A}{A_C} \quad (1) \qquad ^{e}A_f = \frac{^{e}A}{^{e}S} \quad (2)$$

b) Haversian and endosteal radial closure rates ($^{h}M_f$ and $^{e}M_f$): These equal the respective appositional rates multiplied by the decimal fractions of osteoid seams that "took" tetracycline labels. The appositional rate represents a velocity as defined in elementary physics, for it is "a distance divided by time," ie,

$$V = \frac{D}{t.}$$

In this case, the mean tetracycline interband distance is divided by the time interval between the middles of the two markers. Given a 3-10-6 labeling schedule, the time interval between the middle of the first marker band to the middle of the subsequent one equals 14.5 days (3/2 + 10 + 6/2 = 14.5), or .03975 years.

c) Mean cortical thickness (mct): Here this equals the cortical cross-section area divided by the endosteal circumference. Because of the thin cortex of the biopsy samples, obtaining this parameter in this manner introduced only a trivial geometric error.

$$mct = \frac{A_C}{^{e}S} \quad (3)$$

d) The haversian and endosteal bone formation rates ($^{h}V_f$, $^{e}V_f$). These equal the radical closure rate ($^{h}M_f$), multiplied by the osteoid seam density ($^{h}A_f$), multiplied by the mean seam circumference. In symbols:

For haversian bone formation rate:

$$^{h}V_f = {}^{h}M_f \times {}^{h}A_f \times {}^{h}S_f \quad (4)$$

For endosteal bone formation rate:

$$^{e}V_f = {}^{e}M_f \times {}^{e}A_f \times {}^{e}S_f \quad (5)$$

One may express endosteal bone formation in two ways. The *surface-based* rate ($^{se}V_f$) equals the mm² of new bone made per mm² of pre-existing compacta per year.

Thus:

$$^{se}V = {}^{e}M_f \times {}^{se}A_f \times {}^{e}S_f \quad (6)$$

$$^{ev}V_f = {}^{e}M_f \times {}^{ev}A_f \times {}^{e}S_f, \text{ and} \quad (7)$$

$$^{ev}V_f = {}^{se}V_f\,(mct)^{-1} \quad (8)$$

Table II lists the computed data, as well as age-comparable group normals. The group normals averaged the values of 100 normal subjects, taken from a "library" of 327 metabolically normal people of all ages.

Results

1) Cortical area, A_C: This averaged 11.2 mm² + 4.3 mm² per biopsy.

2) Endosteal circumference, ^{e}S: The mean averaged 23.6 mm per biopsy.

3) Circumference of haversian and endosteal seams ($^{h}S_f$ and $^{e}S_f$): These

TABLE I
HISTOLOGIC DATA IN 50 PATIENTS WITH SENILE & POSTMENOPAUSAL OSTEOPOROSIS

		Cortical Area (A_c)	% of Osteoid Seams Labeled	Average Osteoid Seam Circumference (^hS_f)	Osteoid Seam Density per mm² (A_c)
HAVERSIAN ENVELOPE	Osteoporosis	11.2 ± 4.3 (S.E. = .61)	58.2 ± 16.4 (S.E. = 2.3)	0.19 ± 0.05 (S.E. = .0056)	1.11 ± 0.87 (S.E. = .12)
	Normal	13.00 ± 3.20 (S.E. = .32)	95 ± 5 (S.E. = .5)	0.32 ± 0.056 (S.E. = .006)	0.4 ± 0.22 (S.E. = .022)
		Endosteal Circumference (^eS)	**% of Osteoid Seams Labeled**	**Average Osteoid Seam Circumference (^eS_f) in mm**	**Osteoid Seam Density per mm Endosteal Circumference**
ENDOSTEAL ENVELOPE	Osteoporosis	23.6 ± 5.8 (S.E. = .83)	34.5 ± 14.9) (S.E. = 2.1)	0.53 ± 0.20 (S.E. = .29)	0.17 ±0.13 (S.E. = .019)
	Normal	n.a.	95 ± 5 (S.E. = .5)	0.39 ± 0.04 (S.E. = .004)	0.076 ± 0.05 (S.E. = .005)

Table I

The means are listed for the quantitative histological measurements, and compared to norms (where available) for the mean age of the osteoporosis group. One standard deviation and one standard error are also listed for each value.

averaged 0.19mm (H) and 0.53mm (E) per seam, respectively.

4) The haversian osteoid seam density (^hA_f) and endosteal osteoid seam density (^eA_f) averaged 1.11 seams/mm² of compacta, and 0.17 seams/mm of endosteal perimeter, respectively. Group comparable normal values equal 0.41 seams/mm² of compacta and 0.076 seams/mm of endosteal circumference respectively.

5) The haversian radial closure rate (^hM_f) averaged 0.12mm/year compared to the norm of 0.28mm/year; the endosteal radial closure rate averaged 0.075mm/year compared to the norm of 0.23mm/year.

6) The *mean cortical* thickness (mct) equalled *0.47mm*, in contrast to the normal value of *0.80mm*.

7) The volumed-based *harversian bone formation rate* (^hV_f) averaged .030mm² per mm² of pre-existing compacta per year, a 17% decline relative to the comparable normal value of .036mm²/mm² of compacta/year. The median value of the study group lay at 50% of the group's normal (ie, at .016mm²/mm²/.

8) The surface-based *endosteal bone formation rate* (^eV_f) equalled 0.0091mm² per mm² of endosteal surface per year in contrast to the norm of 0.0066 mm² of endosteal surface/year. However the median value equalled 33% of the group's normal, or .0022mm²/mm/year.

The scatter plot in Figure 2 shows formation rates for each case and each envelope in normalized form, and puts a slightly different emphasis on the data than do the arithmetic means. In non-

TABLE II
COMPUTED DATA IN 50 PATIENTS WITH SENILE & POSTMENOPAUSAL OSTEOPOROSIS

		Appositional Rate in μ/day (M)	Radial Closure Rate in mm/year ($^{h}M_f$)	Volume Based Bone Formation Rate ($^{h}V_t$) in mm³/mm³ of compacta/yr.
HAVERSIAN ENVELOPE	Osteoporosis	0.61 ± 0.17 (S.E. = .024)	0.12 ± 0.085 (S.E. = .012)	0.030 ± 0.034 (S.E. = .005)
	Normal	0.90 ± 0.30 (S.E. = .03)	0.28 ± 0.17 (S.E. = .0171)	0.036 ± 0.012 (S.E. =.00036)

		Appositional Rate in mm/yr. (^{e}M)	Radial Closure Rate in mm/year ($^{e}M_f$)	Volume Based Bone Formation Rate in ($^{ev}V_t$) in mm³/mm³/ yr.	Surface Based Bone Formation Rate ($^{es}V_t$) in mm³/mm³ of endosteal surface/yr.
ENDOSTEAL ENVELOPE	Osteoporosis	0.15 ± 0.039 (S.E. = .0055)	0.075 ± 0.049 (S.E. = .0027)	0.019 ± 0.028 (S.E. = .004)	0.0091 ± 0.013 (S.E. = .0019)
	Normal	0.27 ± .1 (S.E. = .01)	0.23 ± 0.04 (S.E. = .004)	0.0082 ± 0.0040 (S.E. = .0004)	0.0066 ± 0.0030 (S.E. = .0003)

Table II
The computed data appear with values for their dispersions and standard covers of the mean. In the osteoporosis group N = 50, and in the normal group it equals 100.

parametric terms 37 (74%) of the 50 subjects had subnormal values for haversian formation, while 34 (68%) of the osteoporosis group had subnormal values for endosteal formation. Therefore, the median, as well as the nonparametric mean formation rates, fell below normal on both envelopes. This finding carries useful statistical significance (ie, $p<.05$).

Discussion

1) *Interpretation:* The study group's mean *haversian bone formation rate* averaged 83.3%of normal, which agrees satisfactorily with previously published tetracycline-based studies of bone formation activity determined in patients after their osteoporosis became clinically apparent .[43] It also agrees with measurements of this activity made by averaging formation over the two decades or so preceding bone biopsy, during which time these patients presumably were developing their disease.[44] Lack of a significant increase in intracortical porosity[18] means that average resorption on the haversian envelope equalled formation, for any excess would have progressively increased this porosity to major proportions during that time.

While the arithmetic mean *endosteal bone formation rate* in this group of patients averaged 150% supernormal when expressed in absolute terms, the scatter plot shows that this represents a distribution effect in which a few individuals with extremely high values

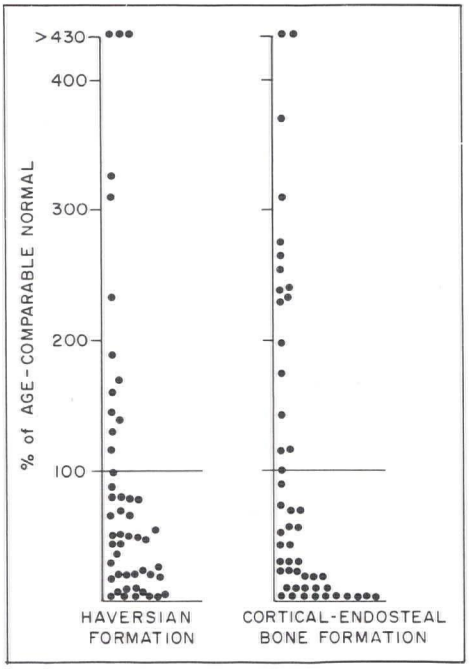

Figure 2
Scatter plots of the formation rates of each case in this study, each value expressed as a percent of the age-comparable norm. Left: haversian formation values. Right: cortical-endosteal values. While a large scatter exists, the preponderance of the values on each envelope lies below normal.

raised the arithmetic mean, although the median value fell significantly below normal, so that the characteristic dynamic state of the osteoporotic patient lay below normal. To explain the enlarged marrow cavities which seemingly typify the osteoporotic skeleton, it must be inferred that endosteal bone resorption exceeds bone formation at supernormal, normal, or even subnormal speeds.

These findings differ only modestly from conclusions reached by Heaney, who believed that organ-level bone formation was normal. The difference may be due to: 1) uncertainty in the size of that fraction of kinetically determined accretion values which reflects histologically measurable bone formation; 2) errors in extrapolating histologically-determined bone dynamic changes in ribs to the rest of the skeleton, presenting findings slightly lower than the true skeletal average or; 3) the still undetermined net contribution of compacta and trabecular bone relative to that of general skeletal dynamics, in normal as well as in osteoporotic individuals.

Similarity of the depressions in haversian and endosteal formation fits the concept that some systemic factor acts on both. However, the presence of a relatively large difference in *net bone loss* on the haversian and endosteal envelopes suggests that some local factor in the marrow cavity may act to increase net endosteal bone loss independently of the status of the bone balance on haversian and periosteal bone surfaces. The marrow cavity is thereby enlarged at the expense of the cortex, a phenomenon which seems to characterize SO and PMO.

2) *Therapeutic Mechanics:* If this is true, lowering the remodeling rate or diminishing the excess of resorption on endosteal bone surfaces (or both) should effectively retard the morphological evolution of this disease. If the normal excess of endosteal resorption relative to formation could actually be reversed we could "cure" SO and PMO! While several diseases exist in which such reversals occur in adult life (making this a possibility), we do not yet understand either the mechanics or the causes of such reversals well enough to devise effective treatment.

3) *Variance:* In the present case material, the coefficients of variation for the formation rates approximated 1.3 for the osteoporotic material and 0.3 for the normal subjects. Numerous other studies indicate that these coefficients represent typical values for quantitative

histological bone work of this type. This variance limited the statistical confidence of previous studies of 18 osteoporotic patients by histological methods,[63] Indeed, the present study reversed the previous one's decrease in the mean endosteal bone formation rate.

4) *Relevance:* Available evidence indicates that any major bone provides reliable clues to the qualitative status of general skeletal dynamics[10,14,16,27,28,30,32,45-50] although remodeling occurs at characteristically different rates in different bones, and even in different parts of the same bone.[26,27,50,51] However, the pattern of these differences becomes stereotyped in different individuals and even in different species; so once sampling sites are standardized, these differences present few problems of interpretation. The human 6th and 11th ribs have adequate reliable tetracycline dynamic standards prepared for comparing normals of all age groups. Such standards are not available for other human bones.[26] Since turnover is quicker compared to most other bones, these ribs reflect characteristic changes sooner and less ambiguously than other bones.

One recent article, questioning the relevancy of ribs to human skeletal dynamics generally,[29] failed to include studies of the whole skeleton and presented new data from only three dogs of unknown age. It provided no statistical analysis of the data presented and did not state the accuracy nor precision of its measurement*. It also used continuous tetracycline labels, which, by suppressing bone formation [26,30] should cause an error in the activity under study.

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*Method appears to be the original method of Frost with two minor modifications, both of which introduce error into determinations of bone formation by depressing bone formation. This is the error which led to the development of the newer method employed in our article.

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