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Evaluation of Cellular-level Haversian Bone Resorption in Human Hyperparathyroid States

A Preliminary Report

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Cellular-level bone resorption was evaluated in 16 patients undergoing renal dialysis and in two with primary hyperparathyroidism, by quantitative histological means using tissue time markers. When averaged over periods greater than two weeks, the individual osteoclasts in these patients resorbed less bone in unit time than normal.

This article reports an evaluation of an index of the speed of cellular-level haversian bone resorption in human subjects with naturally occurring hyperparathyroid states. Such an evaluation has relevance for at least four reasons.

First, direct measurements of this activity in bone have never been reported before, either in human or in experimental material;¹ indeed, some believe it impossible to measure this activity in human material.

Second, many assume that parathyroid hormone makes osteoclasts individually "voracious."¹⁻⁵

Third, humans with naturally occurring metabolic bone disease provide

the actual bone dynamic abnormalities which experimental work must subsequently explain in detail. Such patients have usually been ill long enough by the time of diagnosis and bone biopsy to provide a reasonably reliable steady state with respect to any abnormal bone dynamics that characterize their disease.^{6,7}

Fourth, when imbalance between resorption and formation exists for many months, the bones act as living integrators of this imbalance, showing progressively enlarging and unambiguous morphological consequences. One can measure such changes accurately and directly in bone biopsies, and given the time factor one can express them as rates.

Materials

1) This study includes 16 patients with *chronic renal failure* who were on repeated renal dialysis, and from whom 11th rib biopsies were sent to us by A. G. Shimizu, M.D.; R. G. Yaworski, M.D.; P. Kenmore, M.D.;

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and J. Pierce, M.D. These 4 women and 12 men had *clinical symptoms* of uremia for an average of 1.47 years, and they averaged 36.1 years of age. High levels of circulating parathyroid hormone (PTH) have been found in such patients by others.⁸ All 16 of these patients had clear evidence of renal osteodystrophy, revealed by histological examination of their 11th rib biopsies,⁹ and most of them had had serum chemical evidence of azotemia, and presumably, therefore, had abnormal bone dynamics for much longer than their clinical uremic symptoms.

2) Two women in this study had proven *primary hyperparathyroidism*, and yielded 11th rib biopsies at the time of removal of their parathyroid adenoma.

Table I briefly summarizes the case material.

Methods

1) Labels, Biopsies and Sections:

All patients were given one or more oral tetracycline bone markers of demethylchlortetracycline *in vivo*. Biopsy of the anterior third of the 11th rib was subsequently performed and the specimen was forwarded to the laboratory in 70% ethanol. At least six undecalcified, accurately oriented cross-sections .07mm thick were made upon receipt of the biopsy and stained with the Villanueva osteochrome bone stain, except for one section per case which was not stained. This was to ensure by comparison under the fluorescence microscope that the staining procedures had not eluted and/or obscured the tetracycline labels.^{7, 10, 11}

2) Rationale: The mathematical, histological and physiological relations

TABLE I
Clinical Data on 14 Renal Dialysis Patients

Case Number	Patient's Initials	Diagnosis	Age	Sex	Duration of Symptoms before Rib Biopsy (mos.)	BUN (mg/100ml)	Serum Creatinine (mg/100ml)	Serum Calcium (mg/100ml)	Serum Phosphorus (mg/100ml)	Alkaline Phosphatase (K. A. U.)
1	C. G.	Uremia	30	F	5	146	17.6	10.1	8.96	4.8
2	P. S.	"	30	F	40	225	21.6	8.0	15.6	8.4
3	G. S.	"	47	M	36	n.a.	n.a.	8.5	8.3	n.a.
4	W. B.	"	36	M	5	164	24.4	5.0	11.5	12.0
5	L. V.	"	38	F	7	186	28.4	7.4	7.2	10.8
6	W. H.	"	46	M	25.5	158	22.4	7.8	9.9	10.8
7	E. T.	"	37	F	13	160	16.8	7.5	7.6	7.2
8	R. S.	"	29	M	11	182	23.0	9.5	14.2	4.8
9	E. Y.	"	36	M	26	129	24.0	7.5	8.8	9.6
10	B. B.	"	30	M	3.5	164	18.0	9.3	8.5	14.6
11	A. L.	"	43	M	7.5	215	28.0	8.5	13.7	12.0
12	J. B.	"	42	M	23	145	16.0	4.5	7.45	9.6
13	F. S.	"	48	M	30	103	15.1	9.0	7.2	16.8
14	D. H.	"	30	M	18.5	134	19.4	8.0	6.0	9.0
15	P. H.	"	22	M	12	121	16.7	8.5	7.1	8.6
16	P. M.	"	33	M	20	163	21.0	8.6	8.8	10.4
17	L. H.	i ⁰ HPTH	42	F	24	21	.6	11.2	2.1	5.0
18	E. R.	i ⁰ HPTH	63	F	24	15	1.0	12.1	2.0	4.9

The major clinical features of the case material in the present study are summarized.

Cellular-level Haversian Bone Resorption in Human Hyperparathyroid States

which allow determination of cellular level resorption by quantitative histological means have been outlined elsewhere.¹¹ Based on the use of a minimum of two tissue time markers for formation, and on two more for bone loss or gain, these relations are described next. The basic relation is: When one sums all bone formation and resorption over previous time, their difference equals the amount of bone present. In a modified derivative form, the relation becomes: If the mean bone formation (dF) and resorption (dR) rates are added up during a disease, their difference equals the mean rate of loss or gain (dB) during the disease. In exactly equivalent symbols:

$$dF + dR = dB \quad (\text{Equation 1})$$

Given meaningful values for dF and dB , one then solves this equation for dR . Such values can be obtained by the following means:

dF , the *bone formation rate*, can be measured accurately by two available means.^{7, 11} Here, dB represents the rate of net loss of bone on the haversian envelope during a disease, and any such loss must progressively increase the intracortical porosity, an enlargement easily detected and measured by comparing measurements of this property in biopsies of diseased patients to comparable samples of normal persons. Here, the *normals* reveal the probable status of the patients before they become ill ("marker" #1 for bone loss), while the *patients* reveal any cumulative imbalance that resulted during their disease ("marker" #2 for bone loss).¹³ These differences, divided by the duration of the disease over which they developed, become the mean rate of loss, i.e., dB in equation (1). Converse arguments ap-

ply to net bone gain. Therefore dB is determinate, in histological practice as well as in theory.

Given values for both dF and dB , solving equation (1) for dR yields:

$$dR = dB - dF \quad (\text{Equation 2})$$

Now dR represents the *bone resorption rate*, and in histological terms this also equals the product of that fraction of an envelope's surface undergoing resorption at any moment (R), times the mean depth of bone eroded from that fraction in unit time by osteoclasts (M_r).⁹ Therefore we can "resolve" dR as the product of the above two parameters, thus:

$$dR = R M_r \quad (\text{Equation 3})$$

(R), the *fractional resorption surface*, can also be measured accurately and directly in bone sections. While M_r , the depth of bone eroded in unit time, cannot be so measured, we can now express it as a unique relation to other parameters which can be measured directly, by first substituting equation (3) into (2):

$$R M_r = dB - dF \quad (\text{Equation 4})$$

and then dividing both sides by R :

$$M_r = \frac{dB - dF}{R} \quad (\text{Equation 5})$$

3) **Measurements:** To obtain values for the right hand terms of equation (5), these measurements of haversian remodeling were made by methods outlined in the cited references. The bone formation rate (dF) in units of mm^2 bone formed/ mm envelope perimeter/year,^{7, 11, 15} and the decimal fraction of the total haversian perimeter covered by Howship's lacunae (R).¹⁴ To find dB , the total haversian perimeters in the sections,¹⁶ and the intracortical porosities,¹⁶ were measured.

Haversian, dB: A patient's intra-

cortical porosity was subtracted from his age-comparable norm to obtain the net change (if any) during his disease, subject only to the uncertainty due to normal variation in porosity. One S.D. variation in this porosity represents an amount of bone equal to an 8.2% change in the normal M_r over a period of 1.47 years, which represents a relatively small uncertainty in the value of M_r in an individual case. Positive values of dB imply a deficit, negative values an excess, of resorption relative to formation. For each patient this value was then divided by the duration of his uremic symptoms, to express bone loss or gain as a mean annual rate. When divided by the total haversian perimeter, loss or gain appears as mm^2 of compacta/millimeter of haversian envelope/year.

At this point numerical values were substituted into each term on the right of equation (5), the necessary arithmetic done, and M_r obtained, separately for each case.

4) Normals: Since norms were obtained by similar methods, any system-

atic errors in the work should affect both patients and norms equally. Such errors in any event do not exceed one part in 20, which constitutes the known absolute accuracy of this work in our hands.¹²

Table II summarizes the data along with the norms required for comparison.

Results

1) Haversian Radial Resorption Rate: At $.106 + .1\text{mm/year}$, the mean M_r of the dialysis group averaged 29.5% of the norm for age 36.1 years. The mean of the two women with primary hyperparathyroidism averaged 68% of their mean normal. The scatter plot in Figure 1 shows the distribution of M_r values after expressing the value in each patient as a percent of his age-comparable norm.

2) Evaluation of Reliability: *Dialysis cases*: Equation (5) shows that M_r decreases inversely proportionally to R, and directly proportionally to dF and dB. In the dialysis group (R) was supernormal in 15 patients (aver-

TABLE II
Haversian Envelope

Group N (√N)		Dialysis 16 (4)	Comparable Normal 143 (12)	Primary Hyperparathyroidism 2 (-)	Comparable Normal 81 (9)
Parameter	Units				
Fractional Formation Surface (F)	mm/mm	.04 ± .1	.04 ± .015	.16	.04 ± .015
Fractional Resorption Surface (R)	mm/mm	.185 ± .01	.03 ± .01	.12	.03 ± .01
Rate of bone loss (dB)	mm ² /mm/yr	-.0083	-.0006	-.007	-.0006
Radial Closure Rate (M_r)	mm/yr	.023 ± .02	.34 ± .12	.064	.28 ± .1
Radial Resorption Rate (M_r)	mm/yr	.106 ± .1	.36 ± .1	.15	.22 ± .07

For each patient group, the mean data required to compute the radial resorption rates are listed. These rates were computed separately from the raw data for each case, so the mean radial resorption rate for a given group will not agree exactly with that which is computed from the listed mean values of the raw data.

Cellular-level Haversian Bone Resorption in Human Hyperparathyroid States

age 350% of normal), and slightly subnormal in one patient (90% of normal), so the group's increase in R is significant ($p < .001$) and greatly exceeds the errors of measurement. dF was subnormal in 15 patients (average 34% of normal), and normal in one, so that this depression too is significant ($p < .001$), and it also greatly exceeds the errors of its measurement. dB was elevated in 14 and decreased in 2 patients (average net increase equaled $-.009\%$) and although small, it differs significantly from the normal value of $-.0005 \text{ mm}^2/\text{mm}/\text{year}$ ($p < .01$), and exceeds by a factor of three the errors of its measurement.

Primary hyperparathyroid cases:

In these two women all of the haversian parameters were between one and two S.D. different from normal in that direction which lowered the value of M_r , except the intracortical porosity, which was 3 S.D. larger than normal in each, tending to raise the M_r value. In spite of this, their mean M_r was low, their increased porosities reflecting relatively greater depressions of tissue-level bone formation than of resorption. Although their abnormal changes were at least 10 times larger than the errors of measurement, there are too few of these patients, and their abnormalities are too small, to achieve an acceptable level of statistical confidence.

In sum then, the depressed M_r in the dialysis group is significant, but due to a limited sample that seen in the primary hyperparathyroid women is not. Other factors reinforcing these conclusions will be given shortly.

It is important to note here a dynamic peculiarity of the analytical system that was employed: It averages M_r

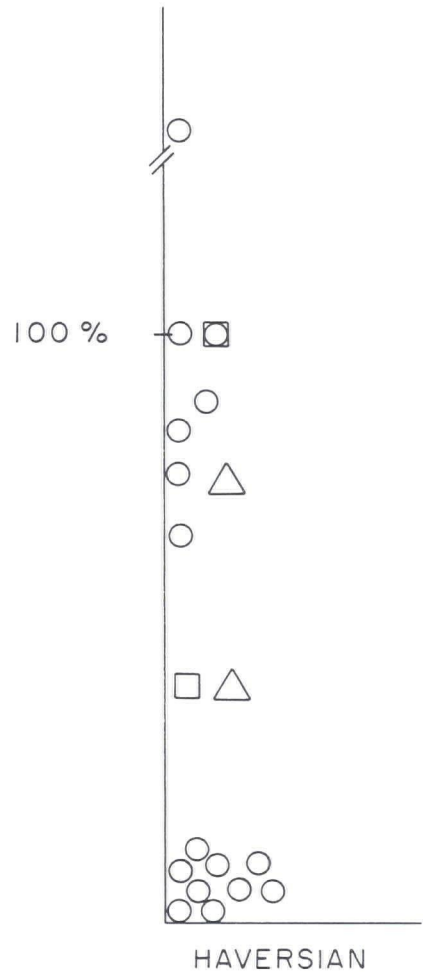


Figure 1

Scatter plot of haversian radial resorption rates, normalized to sex, age- and bone-comparable norms. Circles: dialysis cases. Triangles: primary hyperparathyroid cases. Squares: secondary hyperparathyroidism. The single greatly supernormal haversian value is considered to arise from a greatly underestimated duration of disease. Nevertheless the value of this case was included in computing the dialysis group's mean M_r value.

over time periods equal to or longer than the tetracycline labeling interval, which was approximately two weeks. Consequently these findings do not rule

out the possibility that M_r might temporarily reach normal or even supernormal values over much shorter periods of time, although they do make it very unlikely that the average M_r over longer periods of time was normal or supernormal.

Discussion

A subnormal radial resorption rate alone does not reveal the relative activity of the typical individual osteoclast, that is whether it is supernormal, normal or subnormal.¹¹ However, other information permits these deductions:

First, the fractional resorption surface (R in Table II) was greatly increased in these cases, similar to findings noted by others in similar material.¹⁷⁻¹⁹

Second, and somewhat unexpectedly, the tissue-level haversian bone resorption rates were *subnormal* in the dialysis group, as satisfaction of equation (2) with the data shown ($p < .01$) and as was predicted by others.²⁰ It was normal in the two women with primary hyperparathyroidism (Fig 2).

Third, multiple observers consistently found osteoclasts in greatly increased numbers in these states.^{21, 22} When more osteoclasts lie on an enlarged resorption surface, yet resorb less bone, in terms both of quantity resorbed and of depth eroded in unit time, then *individual osteoclasts resorb bone at subnormal average speed*. The real question then is not whether this is so but rather: how much so?

Two further points enhance the reliability of the above conclusion:

One, these cases had serum chemical

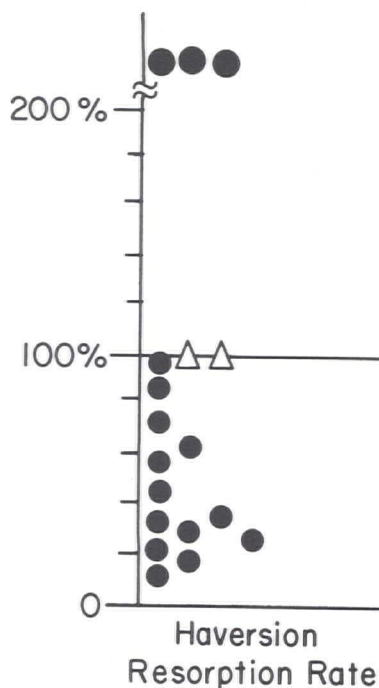


Figure 2

Tissue-level haversian bone resorption rates of each case, normalized to age- and bone-comparable norms. Circles: dialysis cases. Triangles: primary hyperparathyroidism. The three greatly supernormal values are probably spurious, and attributable to underestimating durations of bone disease which, based on clinical symptoms of uremia, were .29, .42 and .47 years. By serum chemical tests all three cases had had azotemia for more than two years, and recomputation on that basis would have brought each of these rates below normal.

evidence of azotemia longer than they had clinical symptoms of uremia, so the estimates of the durations of their bone diseases are probably conservative; this caused the computed M_r values to be higher than the true ones.

Two, some of the increased porosity which was involved in computing dB, and which tends to raise the computed values of M_r , represents a purely tem-

Cellular-level Haversian Bone Resorption in Human Hyperparathyroid States

porary and nonprogressive bone loss which always accompanies increased numbers of bone resorbing and forming centers (average in both of the present groups: 3.5 x normal), each of which adds a temporary deficit of approximately .003 mm² of bone to the envelope on which it lies. This represents a *phase lag pool*.¹¹ Because no correction was made for it, this too caused the computed values of M_r to be higher than their true ones.

In other words, these data define an upper bound of M_r ; the true values

lie somewhere below. For these reasons (as well as others),²³ the depressed M_r in the dialysis group should not arise from sampling effects or from measuring errors.

Clearly we need independent confirmation of these results, particularly in more cases of primary hyperparathyroidism. Clearly too the temptation to ascribe these findings to the effect of parathyroid hormone alone must be resisted, for many other existing factors in each group could have caused them.

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Frost, Villanueva, Jaworski, Meunier and Shimizu

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