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Cellular-Level Bone Resorption in Chronic Renal Failure and Primary Hyperparathyroidism

A Tetracycline-based Evaluation

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The mean depth of bone eroded in unit time at resorption centers was measured in tetracycline-labeled bone biopsies of 16 adult patients on periodic renal dialysis, of one adolescent boy with renal rickets and secondary hyperparathyroidism, and of two adult women with proven primary hyperparathyroidism. This index of cellular-level resorption was subnormal on haversian bone surfaces in all groups, and it was subnormal on cortical-endosteal bone surfaces in the dialysis group; there was insufficient material to evaluate the index on this latter surface in the other two groups. The results suggest that individual osteoclasts resorb bone more slowly than normal in naturally occurring human hyperparathyroid states of long standing.

Key words: Bone — Osteodystrophy — Hyperparathyroidism — Resorption — Osteoclasts.

La profondeur moyenne de l'os érodé, par unité de temps, au niveau de centres de résorption, est musurée sur des biopsies osseuses, marquées à la tétracycline et prélevées au niveau de 16 patients en dialyse rénale périodique, d'un adolescent présentant un rachitisme rénal et de l'hyperparathyroidie secondaire, et de 2 femmes adultes atteintes d'hyperparathyroidie primitive. L'index de résorption cellulaire est sub-normal au niveau des surfaces d'os haversien de tous les groupes. Il est sub-normal au niveau des surfaces osseuses de l'endoste cortical dans le groupe sous dialyse. Le matériel biopsique était insuffisant pour établir l'index dans les deux autres groupes. Il apparait ainsi que les ostéoclastes individuels, provenant de cas d'hyperparathyroidie de longue durée, résorbent l'os plus lentement que dans des conditions normales.

Die mittlere Tiefe von Knochenerosionen, die pro Zeiteinheit in den Resorptionszentren entstanden, wurde in Tetracyclin-markierten Knochenbiopsien gemessen. Die Biopsien stammten von 16 erwachsenen, periodisch hämodialysierten Patienten, ferner von einem Jugendlichen mit renaler Rachitis und sekundärem Hyperparathyreoidismus sowie von zwei erwachsenen Frauen mit erwiesenem Hyperparathyreoidismus. Dieser Index der Resorption auf cellulärer Ebene war in allen Gruppen subnormal, wenn er auf der Innenschicht der Haverschen Kanäle bestimmt wurde. Er war ebenfalls subnormal in der Gruppe dialysierter Patienten auf die cortico-endostale Knochenoberfläche bezogen. Bei den anderen zwei Gruppen konnte der Index für die letztgenannte Oberfläche nicht bestimmt werden, da zu wenig Material vorlag. Diese Resultate lassen vermuten, daß einzelne Osteoklasten in Fällen von langdauerndem Hyperparathyreoidismus beim Menschen den Knochen langsamer resorbieren als dies normalerweise der Fall ist.

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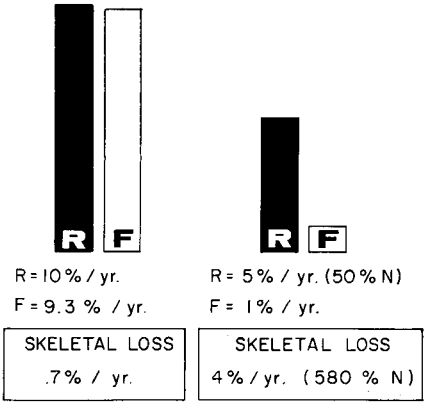

Fig. 1. Left: (Harris and Heaney). Approximately 10% of the normal adult skeleton is resorbed annually and 9.3% is replaced by new bone formation, leaving an average annual net loss between ages 20—60 yrs. of 0.7%. Right: During long-term administration of corticosteroids, and in naturally occurring human hyperadrenalcorticoid states, the bone resorption rate falls, estimated here to half of normal, i.e., 5% per year. However, bone formation almost totally stops, here estimated to proceed at only 1% per year. As a consequence, the net annual skeletal loss rises to 4% per year, more than 5-fold above the normal 0.7%; yet subnormal resorption occurs. (Reproduced by permission: S. Jett, H. Wu, H. Duncan, and H. M. Frost, Adrenalcorticosteroid and salicylate actions on human and canine haversian remodeling; Clin. Orth., 1969)

Introduction

The status of bone resorption at the osteoclast level in naturally-occurring human hyperparathyroid states remains undefined, particularly in chronic renal failure, in secondary hyperparathyroidism due to chronic renal failure, and in primary hyperparathyroidism due to an autonomously functioning parathyroid adenoma. While the possibility exists that parathormone (PTH) makes osteoclasts individually "voracious", contributing thereby to the bone loss and hypercalcemia which may occur in hyperparathyroid states (Best and Taylor, 1961; Neuman and Dowse, 1961; Rasmussen and Tenenhouse, 1967), no direct measurements of cellular-level resorption have been published in either naturally occurring or experimentally induced hyperparathyroid states (Harris and Heaney, 1969; Meuniere *et al.*, 1969).

In this respect, the number of osteoclasts consistently increases in hyper-PTH states (Jaffe *et al.*, 1932; Talmage, 1967), while organ-level bone resorption can increase even if cellular-level resorption decreases, and net bone loss can accelerate even when cellular-level, tissue-level and/or organ-level bone resorption rates decelerate, singly or in any combination (Frost, 1962b, 1964). Consequently, and in the bone system, one cannot infer reliably cellular-level resorptive behavior from organ-level or tissue-level behavior. Fig. 1 shows an example of such a situation, one which in the past has seemed paradoxical to many when presented verbally.

This article presents a partially successful attempt to define the relative status of cellular-level resorption in naturally occurring hyperparathyroid states

Table 1. *Case material. The case material is listed. Mean duration of clinical symptoms of renal failure is given in years in the 6th column*

Case No.	Patient's initials	Age	Sex	Disease	Duration of disease to time of B_x (yrs.)	BUN (mg-%)	Serum creatinine (mg-%)	Serum calcium (mg-%)	Serum PO_4 (mg-%)
1	EG	30	♀	Uremia	0.42	146	17.6	10.1	8.9
2	PS	30	♀	Uremia	3.3	225	21.6	8.0	15.6
3	GS	47	♂	Uremia	3.0	n.a.	n.a.	8.5	8.3
4	WB	36	♂	Uremia	0.42	164	24.4	5.0	11.5
5	LV	38	♀	Uremia	0.58	186	28.4	7.4	7.2
6	WH	46	♂	Uremia	2.1	158	22.4	7.8	9.9
7	ET	37	♀	Uremia	1.1	160	16.8	7.5	7.6
8	RS	29	♂	Uremia	0.9	182	23.0	9.5	14.2
9	EY	36	♂	Uremia	2.1	129	24.0	7.5	8.8
10	BB	30	♂	Uremia	0.29	164	18.0	9.3	8.5
11	AL	43	♂	Uremia	0.62	2.5	28.0	8.5	13.7
12	JB	42	♂	Uremia	1.9	145	16.0	4.5	7.4
13	FS	48	♂	Uremia	2.5	103	15.1	9.0	7.2
14	DH	30	♂	Uremia	1.5	134	19.4	8.0	6.0
15	PM	33	♂	Uremia	1	151	22.2	8.7	7.9
16	PH	22	♂	Uremia	1.6	190	27.4	9.1	5.7
17	LH	42	♀	1° HPTH	2	15	0.8	12.2	2.2
18	MR	63	♀	1° HPTH	2	12	0.5	11.9	2.1
19	DG	14	♂	1° HPTH (+2° HPTH)	10	90	8.0	8.5	10.1

by quantitative histological means, in terms of the *radial resorption rate* (M_r), an index of the activity which represents the opposite number of the bone appositional rate.

Materials

1. The 16 renal failure patients have been the subjects of previous studies of cellular and tissue-level bone formation and of tissue-level resorption (Hitt *et al.*, 1969; Sarnsethsiri *et al.*, 1969; Villanueva *et al.*, 1969). There were four women and 12 men of mean age 36.1 years. The mean duration of their clinical symptoms of uremia was 1.47 years, and Table 1 lists the duration for each case. In most cases this time period was shorter than the duration of serum chemical and urinary evidence of renal insufficiency and azotemia, so that the durations of their abnormal bone dynamics, which we defined arbitrarily as the duration of clinical symptoms of uremia, are almost certainly underestimated, a point which will assume some importance later. While a more rigorous estimate of this duration would be desireable we could not obtain one.

Table 1 lists the salient clinical features of these patients. All had well advanced uremic or azotemic osteodystrophy on histological examination of their bone biopsies, and such patients usually have high levels of PTH circulating in their blood (Berson and Yalow, 1966). One of these patients, case 10, had clear radiographic evidence of secondary hyperparathyroidism when he was first seen at the Hamilton dialysis center, 0.29 years before his rib biopsy. These cases were submitted through the generosity of A. G. Shimizu, M. D., and R. G. Yaworski, M. D. both of Hamilton, Ontario; Peter Kenmore, M. D., of Washington, D. C., and James Pierce, M. D., of Grand Rapids, Mich.

2. Two cases of primary hyperparathyroidism due to proven parathyroid adenoma, and with typical serum chemical changes but without radiographic evidence of skeletal involvement, as well as one 14-year-old boy with severe rickets and typical *secondary hyperpara-*

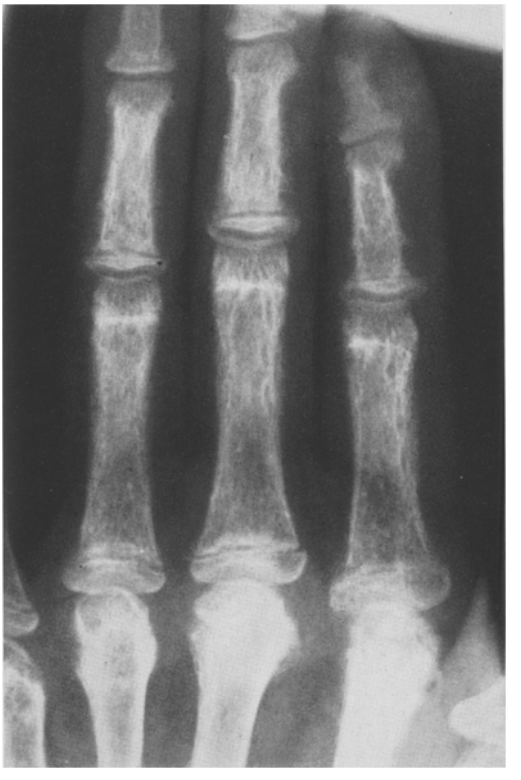

Fig. 2. X-rays of the hands of the 14-year-old boy with renal rickets and secondary hyperparathyroidism in the case material of this report. The subperiosteal erosions characteristic of this state show clearly and represent a periosteal "decoupling" of resorption from formation, leaving the former in relative excess but not of itself revealing whether the absolute value of the resorption rate is increased, normal or decreased. The findings of this study imply a decrease

thyroidism due to chronic renal disease, were seen and subjected to bone biopsy at Henry Ford Hospital. Table 1 also lists their salient clinical features, and the X-rays of the boy are shown in Fig. 2. One of these cases was submitted by D. C. Mitchell, M.D., of Henry Ford Hospital.

Methods

1. Labels, Biopsies and Sections

Each patient in this study was given two oral demethylchlortetracycline bone markers, each of three days duration and separated by 10 days. Continuous markers were avoided to minimize any effect of the marker on the activities under study (Hong *et al.*, 1968; Saxen, 1966). From one to three weeks later an 11th rib biopsy was done. An exception was case 19, who had bilateral corrective pertrochanteric osteotomies; a transverse section of the proximal femoral metaphyseal cortex was removed at each of these operations. Forwarded to the laboratory in 70% ethanol, hand-ground, accurately-oriented complete cross-sections were made and stained with the Villanueva Osteochrome stain (Ramser *et al.*, 1966). The sections averaged 0.07 mm thick and at least six were made per case, one of which was not stained to ensure that the staining procedure had not eluted or obscured any tetracycline labels.

Both elution and obscuration by stain may occur of recently deposited labels, or of labels in osteomalacic persons, particularly if their biopsies are done too soon after the label, or if the specimen is left too long in solutions or embedded in plastic (Ramser *et al.*, 1966).

2. *Rationale*

a) Envelopes. Since bone remodeling can change differently on haversian system and cortical-endosteal surfaces in aging and in some diseases, implying surface-specific dynamic effects (Frost, 1966; Jett *et al.*, 1969; Wu *et al.*, 1967), these two surfaces were evaluated separately in this work. In the generic sense henceforth, they will be called the *haversian envelope* and the *cortical-endosteal envelope*, while specific measurements of either will be referred to as perimeters.

b) Definition of the Index. The cellular-level resorption rate represents the quantity of bone resorbed per osteoclast (or better, per osteoclast nucleus) in unit time. The mean depth of bone eroded in unit time at surfaces undergoing resorption can provide an index of this rate. Analogous to the thickness of ice melted on a skating rink in unit time, M_r will signify this index here.

c) The Basic Equation. This equation, when satisfied with numerical values, will provide M_r:

$$M_r = \frac{dB - FM_f}{R} \tag{1}$$

where M_r, the unknown to be determined and named the *radial resorption rate*, represents the mean annual thickness in mm of bone resorbed at resorption foci; dB signifies the mean annual mm^2 (as seen in cross-section) of gain or loss of bone per mm perimeter of an envelope, due to excess of formation over resorption or *vice versa*; F (the *fractional formation surface*) and R (the *fractional resorption surface*), designate the decimal fractions of an envelope perimeter which osteoid seams or Howship's lacunae cover, respectively; M_f, the *radial closure rate*, expresses the mean thickness of new bone deposited annually on the average unit area of osteoid seam, determined with the aid of tetracycline markers.

d) Derivation of Eq. (1). Simple histological, geometric and mathematical relations permit histological determination of organ-level, tissue-level and cellular-level bone resorption rates as follows (Frost, 1962a; 1964, equations *4.03, 4.16, 5.11, 5.12, 5.16, 5.17, 6.08, 6.19, 6.31;* Wu *et al.*, 1967): When all bone Addition (A) and Subtraction (S) over all past time are added, their net difference equals the amount of bone present at the moment of observation (B).

In exactly equivalent symbols and noting that S signifies a minus quantity:

$$A + (-S) = B. \tag{2}$$

Two modifications adapt this relation to the present problem: when the mean *rates* of Addition and Subtraction *during a disease* are added up, any difference will represent the mean rate of bone gain or loss during the disease. Again, in exactly equivalent symbols:

$$dA + (-dS) = dB. \tag{3}$$

Note that if dS exceeds dA, dB, will take a negative value signifying bone loss, while if the opposite is true, dB will take a positive value signifying bone gain. If dA equals dS, then formation equals resorption, dB will equal zero, and no bone gain or loss occurs. Given meaningful values for dA and dB, then solving Eq. (3) will provide $(-dS)$, the resorption rate. In symbols:

$$-dS = dB - dA. \tag{4}$$

The following paragraphs outline our approach to obtaining values for dA and dB, and also a method of "resolving" $(-dS)$ to obtain finally the radial resorption rate, M_r.

Obtaining dA. One can measure this directly (it simply represents the *bone formation rate*), either by Frost's (1963) original method, recently used in modified form by others (Harris and Heaney, 1969), or by the present method as the mean bone forming surface per unit

perimeter of an envelope (F), multiplied by the mean thickness of new bone deposited on it annually (M_f) (Frost, 1968). In symbols then:

$$dA = F\,M_f. \tag{5}$$

Substituting this into Eq. (4) yields:

$$-dS = dB - F\,M_f. \tag{6}$$

While others call F the percent formation surface (Jowsey *et al.*, 1965; Meuniere, 1968), the fractional formation surface more correctly signifies it.

Obtaining dB. Obtaining the mean rate of bone gain or loss during the disease (i.e., dB in equations 4, 6) requires two data for each envelope and case; *the net gain or loss during the disease* (B), and the *duration of disease* (t) over which gain or loss (if any) occurred. Estimation of B was based on these facts: during a disease a constant excess of haversian resorption over formation will progressively enlarge the intracortical porosity, while a constant excess of cortical-endosteal resorption over formation will progressively enlarge the marrow cavity; one can measure both of these properties directly and accurately in bone biopsies which provide a complete cross-section of the marrow cavity. Since bone acts as a biological and morphological integrator, the longer such excesses exist before biopsy, the greater and easier to measure become any enlargements they cause in porosity and marrow cavity area. The converse holds equally true when formation exists in excess.

Then by subtracting the cortical porosity and marrow cavity size of each patient from a comparable norm, the net gain or loss during his disease can be estimated, subject to the errors of measurement, (which are trivial here) and to the not-so-trivial uncertainty introduced by the normal interperson variance in these two features, of which more later in this article. When divided by the duration of disease, obtained of necessity by history, we then obtain the mean rate of gain or loss in unit time i.e., dB, for each envelope. While admittedly bone loss may have occurred at different rates during the early and later stages of the disease, we could not evaluate this from a quantitative standpoint and so settled for the assumption, implied in the above arithmetic procedures, that it was linear.

For the *haversian envelope*, the mean intracortical porosity of each case, subtracted from the interpolated age- and bone- comparable norms shown in Table 4, and then divided by the duration of uremic symptoms in years, (t), yielded the change in porosity as a mean annual rate; when also divided by the total mean haversian perimeter in the average mm^2 of cortical cross-section area (^hS, *haversian canal specific surface*, Table 4) dB appeared in units of the mean mm^2 of compacta gained or lost, per mm of haversian canal perimeter, per year. Symbolically:

$$^hdB = B\,(^hSt)^{-1} \tag{7}$$

Table 2 lists these haversian dB data.

The normal 2% net increase in intracortical rib porosity between ages 20—60 years (Epker *et al.*, 1965) posed no problem here because it averages an annual net loss of 0.0005 mm^2 of compacta/mm haversian perimeter, which falls nearly two orders of magnitude below the normal annual haversian bone resorption rate. The normal interperson variance (i.e., $\pm$ 1 S.D.) in intracortical porosity represents an amount of bone equivalent to a 12% change for a period of one year in the normal adult M_r. While not trivial, this still introduces a small uncertainty into any one patient's value for M_r. Therefore, lacking other disturbing factors, the haversian M_r in any given case should receive considerable weight, understanding that a meaningful change in any one individual represents a difference from his norm by two or more S.D.

For cortical-endosteal dB (i.e., bone gain or loss from the marrow cavity walls) divide the marrow cavity cross-section area by the total cross-section area to obtain the M/T ratio, or marrow cavity size as a decimal fraction of the total volume of the rib biopsy. This normalizes the data for some of the variation associated with differences in body size and height (Sedlin, 1964b). The M/T ratio for each case then was subtracted from interpolated age-, sex- and rib comparable norms in Table 4, the difference- if any- converted back to mm^2 of compacta by multiplying by the patient's total cross-section area and then divided, first by the duration

Table 2. *The haversian envelope data is given for each case. F: fractional formation surface lacunae/mm envelope perimeter. Porosity: the fraction of the compacta occupied by all vascular channels in mm²/mm². dB: gain (plus) or loss (minus) of bone during the disease as mm² as mm thickness of bone eroded/year, averaged*

Case number Initials	1 EG	2 PS	3 GS	4 WB	5 LV	6 WH	7 ET	8 RS	9 EY
Haversian									
F	0.04	0.09	0.48	0.80	0.35	0.45	0.04	0.09	0.05
R	0.09	0.20	0.21	0.01	0.57	0.26	0.16	0.05	0.22
Porosity	0.048	0.068	0.042	0.065	0.067	0.054	0.045	0.047	0.066
dB	−0.008	−0.0029	−0.0003	−0.022	−0.017	−0.003	0	−0.003	−0.0048
M_f	0.016	0.006	0.006	0.012	0.010	0.007	0.019	0.018	0.049
M_r	0.096	0.017	0.015	2.2	0.035	0.023	0.0042	0.092	0.032

Table 3. *The cortical-endosteal envelope data is listed for each case, the symbols having the area divided by the mean*

Case number initials	1 EG	2 PS	3 GS	4 WB	5 LV	6 WH	7 ET	8 RS	9 EY
Cortical endosteal									
F	0.094	0.44	0.47	0.77	0.48	0.60	0.19	0.29	0.24
R	0.16	0.29	0.22	0.04	0.12	0.08	0.14	0.07	0.09
M/T	0.36	0.41	0.46	0.56	0.49	0.65	0.50	0.75	0.65
dB	+0.30	+0.019	+0.0052	0	−0.022	−0.043	−0.024	−0.30	−0.056
M_f	0.018	0.012	0.003	0.008	0.006	0.006	0.015	0.051	0.027
M_r	0	0	0	0.15	0.21	0.58	0.19	4.4	0.69

of disease, (t), and second by the total cortical-endosteal perimeter (${}^{c}S$), to obtain loss or gain as mm² of bone per mm of cortical-endosteal perimeter per year. Symbolically:

$$ {}^{e}d\,B = {}^{e}B\,({}^{e}St)^{-1}. \tag{8}$$

These data appear in the endosteal dB row in Table 3. The normal interperson variance in M/T ratios (i.e., ± 1 S.D.), relative to both the normal cortical-endosteal resorption rate and M_r, represents 10 times more bone than that normally resorbed annually on the entire cortical-endosteal surface. Accordingly individual values for cortical-endosteal M_r receive low weight.

Resolving dS. At this point we can satisfy Eq. (6) to obtain $-dS$, the *bone resorption rate*, expressed as mm² of bone removed, per mm of envelope perimeter, per year. Now, in theory as well as in practice, this rate also equals the mm of resorption surface per mm perimeter of an envelope (R), multiplied by the mean depth of bone eroded per mm perimeter of this surface annually (M_r) (Frost, 1964), thus:

$$ -d\,S = R\,(-M_r). \tag{9}$$

Since we can measure (R) directly in undecalcified bone cross-sections (Wu *et al.*, 1967), and since we now know $-dS$, M_r can appear as a relation to these other two parameters, thus:

$$ -M_r = -d\,S/R. \tag{10}$$

in mm osteoid seam/mm envelope perimeter. R: fractional resorption surface in mm Howship's bone/mm envelope perimeter/year. M_f: the radial closure rate, as mm. thickness of new bone deposited/year, averaged over the whole osteoid seam perimeter. M_r: the radial resorption rate, over the whole Howship's lacunar surface

)	11	12	13	14	15	16	17	18	19
B	AL	JB	FS	DH	PM	PH	LH	MR	DG
0.46	0.13	0.38	0.33	0.05	0.058	0.11	0.07	0.26	0.56
0.07	0.18	0.39	0.33	0.04	0.092	0.047	0.05	0.20	0.31
0.079	0.051	0.031	0.053	0.033	0.054	0.063	0.081	0.090	0.21
0.057	−0.02	+0.004	−0.007	+0.003	−0.005	−0.008	−0.007	−0.007	−0.006
0.030	0.012	0.040	0.008	0.10	0.017	0.024	0.084	0.044	0.46
1.0	0.019	0.28	0.029	0.05	0.065	0.22	0.24	0.09	0.84

same meaning and units as in Table 2. The M/T ratio is the mean marrow cavity cross-section total cross-section area

)	11	12	13	14	15	16	17	18	19
B	AL	JB	FS	DH	PM	PH	LH	MR	DG
0.69	0.48	0.76	0.41	0.10	0.14	0.28	0.31	0.25	0.33
0.08	0.08	0.21	0.22	0.41	0.12	0.28	0.18	0.22	0.20
0.69	0.45	0.56	0.63	0.42	0.51	0.50	0.57	0.51	—
0.67	+0.27	+0.0069	−0.021	+0.017	+0.0065	−0.0082	−0.059	+0.019	—
0.021	0.011	0.013	0.005	0.024	0.014	0.044	0.13	0.066	0.41
.5	0	0	1.05	0	0	0.072	0.55	0	—

If we substitute Eqs. (9) into (6):

$$R(-M_r) = d\,B - FM_f, \tag{11}$$

and then solve this expression for M_r by dividing both sides by R, we obtain Eq. (1).

e) Interpretation of M_r. By the strategy just outlined and with certain qualifications referred to in the Discussion part of this article, a supernormal M_r implies individually hyperactive osteoclasts and a subnormal M_r implies individually hypoactive or "lazy" osteoclasts.

3. Measurements

Here the millimeter will serve as the unit of length, the year of time and rates will appear on an annual basis.

a) Perimeters. The total *osteoid seam* and *resorption cavity perimeters*, as well as the total haversian and cortical endosteal *envelope perimeters*, were measured in each section with a point-count grid method described previously (Chalkley, 1963; Frost, 1962a; Weibel, 1963; Wu *et al.*, 1967; Zeiss, 1960). Accuracy and precision of measurement relative to the mean value per section, equalled one part in 30. The seam and resorption cavity values, when divided by the total respective envelope perimeters, equalled the *fractional formation surface* (*F*), *and the fractional resorption* (*R*). Moving the decimal two places to the right yields the percent formation and resorption surfaces. Tables 2 and 3 show these data.

Table 4. *Normal histological values M/3 11th human rib. Norms, which were used to evaluate the experimental data in Tables 2 and 3, listed by decades. The haversian canal specific surface is the mean amount of haversian canal perimeter per mm² of cortical cross-section area. The gain or loss of compacta in the experimental material, which is indicated by a decrease or enlargement in their intracortical porosities, occurs on this amount of haversian canal perimeter, so these gains or losses were divided by the specific surface to obtain them in units of mm² of compacta/mm of haversian perimeter*

Mean age	15	25	35	45	55	65
Age range	10—19	20—29	30—39	40—49	50—59	60—75
Number of subjects	20	49	52	53	63	47
Haversian canal specific surface (h_s) (mm/mm²)	2.4 ±0.57[a]	2.1 ±0.33	2.2 ±0.55	2.4 ±0.48	2.5 ±0.42	2.4 ±0.42
Cortical porosity due to vascular channels (mm²/mm²)	0.030 ±0.004	0.037 ±0.004	0.043 ±0.004	0.048 ±0.005	0.054 ±0.006	0.058 ±0.006
Haversian radial resorption rate (h_{M_r}) (mm/yr.)	2.1	1.6	0.38	0.30	0.22	0.23
Cortical-endosteal radial resorption rate (e_{M_r}) (mm/yr.)	0.70	0.55	0.50	0.41	0.32	0.22
M/T ratio, 11th rib, men	0.47	0.52	0.56	0.58	0.60	0.61
M/T ratio, 11th rib, women	0.43	0.45	0.47	0.49	0.52	0.55

[a] one standard deviation.

b) Radial Closure Rate (M_f). This was found on each envelope, with the fluorescence microscope and a calibrated eyepiece micrometer, by measuring in each section the mean distance between every pair of tetracycline labels, dividing by the time in years between them, and averaging over the envelope's whole osteoid perimeter (Villanueva *et al.*, 1969). Accuracy of the mean value of this parameter per section equalled ±0.01 mm/yr and precision was ±0.004 mm/yr. Tables 2 and 3 show these data.

c) Areas. The intracortical porosity, or fraction of compacta occupied by vascular channels, was measured under medium magnification in each section of each case with the Zeiss integrating eyepiece I to an accuracy, relative to the mean per section, of ±0.001 mm²/mm² (Chalkley, 1943; Frost, 1962a; Zeiss, 1960). Table 2 shows these data.

The *marrow cavity and total cross-section areas* were measured in each section with a point-count grid method described elsewhere (Frost, 1962; Sedlin, 1964a) to an accuracy per section of ±0.3 mm². Table 3 shows these data.

4. Norms

Table 4 lists the essential norms, the normal interperson dispersions as ± one standard deviation, and the number of individuals studied in each normal age decade (i.e., N; Jett *et al.*, 1967; Pirok *et al.*, 1966).

Results

1. Haversian Radial Resorption Rate (M_r)

The mean for the *dialysis group* equalled 0.106 mm/yr compared to a group norm of 0.37 mm/yr ±0.19 (S.E. 0.025). However this rate was subnormal in 14 of the dialysis cases; it was normal or supernormal in cases 10 and 4 respectively, both of whom had clinical symptoms of uremia for only 0.29 and 0.42 years respectively prior to rib biopsy. If, as seems probable, their abnormal bone

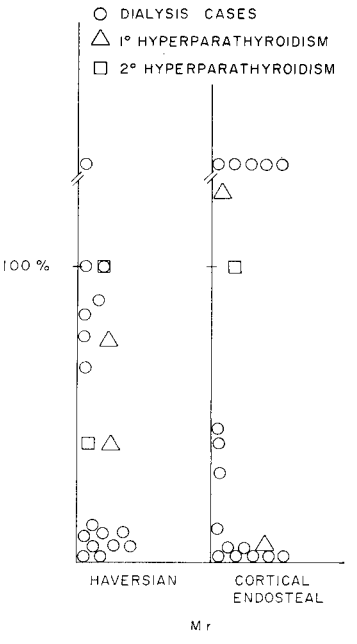

Fig. 3. Scatter-plot of M_r values, for the haversian envelope on the left, the cortical-endosteal envelope on the right. Each value was normalized with respect to 100% of the age- and bone-comparable norm, the latter determined in the same way as the experimental values. On this scale, and other things being held the same, one interperson S.D. dispersion in intracortical porosity represents approximately 12% of the normal annual haversian M_r, while for normal marrow cavity cross-section areas, it represents more than 100% of the normal cortical-endosteal M_r value

dynamics existed longer than this (case 10 had clear radiographic evidence of secondary hyperparathyroidism when first seen at the dialysis center, which certainly took longer than 0.29 years to develop) their true values of M_r would be reduced in inverse proportion. If we eliminate case 4 and recompute M_r for the remaining 15 dialysis cases, the mean of 0.031 mm/yr $\pm$0.02 (S.E. 0.004) equals only 7.9% of the age-comparable norm and represents a profound and highly significant depression ($p < 0.001$). The observation that he did not have unusually large or numerous resorption cavities, although he should have if his computed M_r of 2.2 mm/yr was his true rate, further justifies elimination of case 4. Note that such elimination does not alter the nature of the group findings; it only modifies their magnitude.

Calculation of M_r depends on the thickness of new bone being deposited in unit time (i.e., M_f), which on both envelopes and in every case was greatly and significantly subnormal, the group mean falling below 7% of the group norm ($p < 0.001$; Sarnsethsiri *et al.*, 1969). The scatter plot in Fig. 3 shows these data (circles) after normalizing each case to 100% of his age-comparable norm. Clearly, as a group the average unit area of resorption surface was eroded at greatly subnormal speed.

In the boy with renal rickets and *secondary hyperparathyroidism* (square in Fig. 3), M_r at 0.84 mm/yr, represented 41% of his norm of 2.1 mm/yr. Case 10, the adult dialysis patient with secondary hyperparathyroidism, appears in Fig. 3 as a circle within a square, and has a normal computed value. However if recomputed on the basis that he had had his illness for three years (which was true on serum chemical grounds), his M_r falls slightly below one-ninth of normal.

In the two women with *primary hyperparathyroidism* (triangles in Fig. 3) this rate equalled 75% of normal in one and 39% of normal in the other.

2. *Cortical-endosteal Radial Resorption Rate (^eM_r)*

In the *renal failure patients*, this rate fell below normal in 11 and above it in 5, two of which were cases # 8 and, again, # 10. The mean M_r for the whole group fell moderately above normal at 0.62 mm/yr ±1.4. However, if recomputed without the enormously and almost certainly spuriously supernormal rates of cases 8 and 10, M_r equals 0.17 mm/yr ±0.08 (S.E. 0.02), or 34% of the age-comparable norm of 0.50 mm/yr ±0.25 (S.E. 0.033, $p < 0.05$). Eliminating cases 8 and 10 can be justified further by the observation that at M_r values of 4.4 and 8.5 mm/yr respectively, it would take less than 4 months to completely resorb through the cortex of their ribs, which averaged a normal 0.55 mm in thickness. Net bone loss of this magnitude does not occur in clinical practice in renal dialysis cases.

As Eq. (1) shows, the value of cortical-endosteal M_r in any one person depends greatly on his actual marrow cavity cross-section area. Even after normalization the coefficient of variation for normal interperson variance in M/T ratio equals 0.25 (Sedlin, 1964b) which in absolute units represents ±0.14 mm² of cortical bone/mm of cortical-endosteal perimeter. This exceeds by a factor of approximately 9 the normal interperson variance in haversian porosity (which in absolute units equals ±0.15 mm² of cortical bone/mm haversian envelope perimeter). Accordingly, in any single case haversian M_r provides a nine times more sensitive and dependable indication of cell-level resorption than does the cortical-endosteal M_r; the *group* mean of the latter provides the only acceptable clue to the dynamic state which characterizes the disease.

With respect to the distribution of marrow cavity sizes, 9 dialysis cases had smaller, 7 larger marrow cavities than bone-, age- and sex-comparable predicted normals, which suggests that no unusual enlargement of the marrow cavity occurred in the group during their disease; if anything, a slightly subnormal rate of enlargement is suggested, the normal annual loss between ages 20—60 yrs. [i.e., dB in Eq. (1), and Table 3] averaging only —0.006 mm² of compacta/mm cortical-endosteal envelope/year in this bone.

Note here that the 3.5 times supernormal numbers of bone remodeling centers on the cortical endosteal surfaces cause a fixed non-progressive increase in marrow cavity size, since each of these centers represents an average but purely temporary bone deficit of ≈ 0.003 mm². This property exists apart from any progressive net losses of bone that might characterize a disease, and causes computed M_r values to fall above the true values. This property represents a *phase-lag pool*, discussed elsewhere as a property of complex dynamic systems (Frost, 1964).

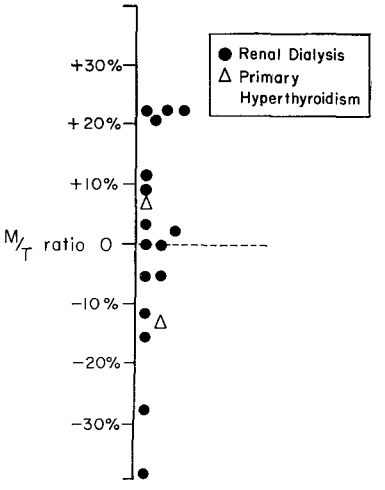

Fig. 4. The M/T ratios of all cases (except the boy with renal rickets), each value normalized to 100% of the age-, sex-, and bone-comparable norm. This plot suggests a normal distribution of marrow cavity sizes in the dialysis cases (circles) with the mean and median values slightly but not significantly over on the small marrow cavity side (i.e., values above the 100% line)

Fig. 4 shows the M/T ratios as a scatter plot, which expresses each case as a percent of the bone-, age- and sex-comparable normal ratio. This scatter plot reveals an essentially normal distribution of values (Santoro and Frost, 1968; Sedlin, 1964a; Takahashi and Frost, 1966).

In the boy with *renal rickets*, we cannot reliably estimate cortical-endosteal M_r because no normal standard exists for marrow cavity size at his sampling site (proximal femoral metaphysis).

In one of the 2 cases with *primary hyperparathyroidism* M_r fell modestly above normal (i.e., 120%) but probably spuriously so, since her much more reliable haversian rate was subnormal and the normal variance in marrow cavity size means that the probability that her elevated endosteal M_r was due to a sampling effect lies between 0.3 and 0.5. In the other woman this rate fell below zero which certainly was a sampling effect because her marrow cavity was smaller than normal. The mean of these two women, while not statistically meaningful, equals 63% of their group norm.

Discussion

Spatially speaking, the parameter defined in this work, M_r, signifies the mean thickness of bone eroded in unit time, averaged over the whole resorption surface. Dynamically speaking, an inherent property of our methods averaged the value of M_r over a period longer than 2 weeks, partly because this was the usual time between the centers of the two tetracycline bone markers. Within these contexts, M_r fell significantly below normal in the dialysis group, and suggestively so in each of the other three patients. On the cortical-endosteal envelope M_r fell below normal at borderline significance in the dialysis group but because of

sampling problems it could not be estimated reliably in the other three patients. A subnormal M_r implies hypoactive osteoclasts.

Because the methods averaged M_r over time periods of two weeks or longer, a subnormal value does not preclude the possibility of normal and/or supernormal values over much shorter time periods, i.e., that osteoclasts might become hyperactive for brief periods in these diseases. Certainly hyperactivity can occur *in vivo* for brief periods following the introduction of parathormone into an organ-culture system (Gaillard, 1961). However, subnormal M_r values do preclude the possibility of continuously supernormal, or average normal or supernormal, activity over two weeks or longer time periods.

With respect to the reliability of the above conclusion, the four following points apply:

1. All three of these groups of patients had been ill long enough to attain, or at least to approach better than any short term experimental situation, the steady state with respect to any endocrine or other regulatory actions on their bone-cell behavior which might characterize their illness.

2. They also had been ill long enough to accumulate large bone morphologic changes from any large imbalances between resorption and formation associated with their diseases. For example, a large increase in resorption without a similar increase in formation, would cause glaringly obvious increases in intracortical porosities and marrow cavity sizes after 1.47 years.

3. In the dialysis group, underestimating the actual duration of the abnormal bone dynamics, as well as not correcting for the purely temporary but nonprogressive losses of bone which always accompany increased *numbers* of remodeling centers, (i.e., the phase lag pool mentioned earlier) would raise the values of M_r somewhat above the true ones.

4. Finally, it might be assumed that resorption in these patients was actually increased in direct proportion to the increases in resorption surfaces, a view which others have advocated (Jowsey and Raisz, 1968; Jowsey *et al.*, 1965). The resorption surfaces were enlarged 3- to 4-fold above normal on both haversian and cortical-endosteal envelopes. Since the normal mean haversian resorption rate at age 36 years equals 0.019 $mm^2/mm^2/yr$, or 1.9% of the compacta annually (Pirok *et al.*, 1966), a 3-fold elevation would equal 5.7% per year. Offset this by their bone formation rate of 0.6% per year, which represents 34% of their norm (Pirok *et al.*, 1966). Therefore the assumed annual excess of resorption over formation would equal 5.1% which, when added over 1.47 years to their presumably normal original porosity of approximately 5%, should have raised it to 12.1%. Yet the actual mean haversian porosity of the group equaled only 5.5%. The difference between the actual and this devil's advocate- predicted value exceeds the errors of measurement by a factor of 30. Therefore resorption could not average 3-fold or more above normal in these patients.

We cannot yet convert M_r values to resorption per osteoclast (i.e., an absolute definition of cellular-level resorption, or zeta in equation (6.19) in Frost's monograph (1964), because (to our knowledge) nobody has quantified the number of these cells — or better, the number of their nuclei per unit area of resorption surface in these diseases. However, three other facts, in conjunction with these data, allow one to infer the status of this activity relative to normal.

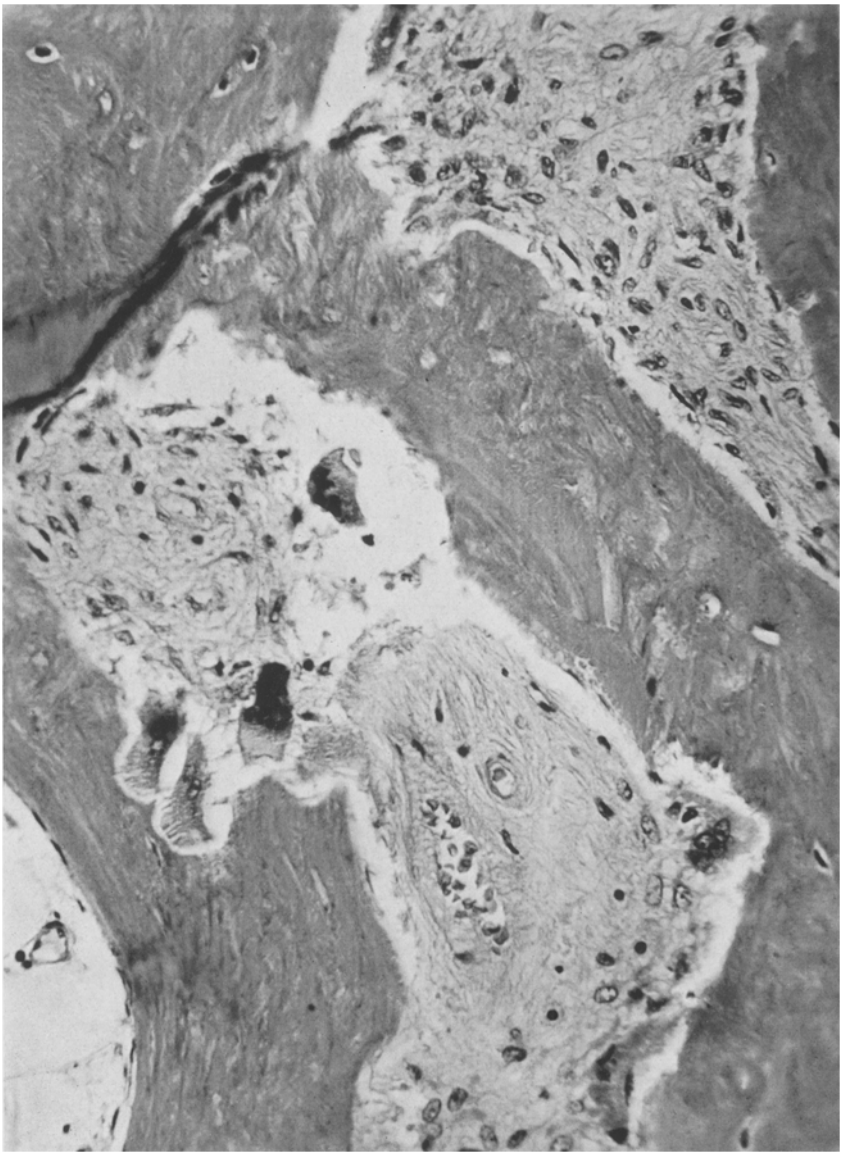

Fig. 5. Photomicrograph of vertebral spongiosa from a woman dying from severe (but clinically unrecognized) hyperparathyroidism. Numerous and greatly supernormal numbers of osteoclasts lie in the many resorption days. Since the appositional rate falls greatly and consistently below normal in these states (≈ 0.1 μ/day or $\approx .036$ mm/yr), eliminating the possibility of equally rapid replacement (Tables 1 and 3), the presence of trabeculae of the thickness shown might alone suggest poisoning of these cells by some chemical messenger(s) in the blood

First, increased numbers of osteoclasts consistently occur adjacent to resorption surfaces in bones from such patients (Jaffe *et al.*, 1932; Meuniere, 1968, 1969; Talmage, 1967), and a photomicrograph of such an increase in a case of our own

appears in Fig. 5. Trabeculae are on the order of 0.08 mm thick, from which it is clear that if (typically) osteoclasts eroded as much bone per day as the 100 to 1,000 μ proposed by Johnson (1964) or reported by Gaillard (1961), affected trabeculae would disappear entirely within a few hours. Second, the tissue-level resorption rates in all but one of the present cases fell significantly below normal, as Kaye and Silverman (1965) postulated might occur (Villanueva *et al.*, 1969). Third, the amount of resorption surface (i.e., *R*) was increased three to four-fold in our case groups, and was supernormal in every patient in this study except case 4, who had slightly subnormal values on both envelopes; others have resorted similar findings in similar material (Meuniere, 1968; Meuniere *et al.*, 1968).

These facts convey a clear message: increased numbers of osteoclasts, which nevertheless erode subnormal quantities and depths of bone in unit time, means that *individual osteoclasts resorbed less bone than normal.* The real question then becomes not the relative one of whether, as individuals, these cells resorbed bone too slowly but the absolute or quantitative one: "exactly how slowly did they do so?" The possibility must be kept in mind that some other, as yet unidentified but still important factor in hyperparathyroid states could participate in causing such changes. Dent (1962) alludes to this possibility and Frost *et al.* (1968) suggested such a factor associates in some way with the kidney.

These results require independent confirmation (now under study by P. Meuniere in Lyons), and additional cases need to be studied, particularly of primary hyperparathyroidism. Appropriate questions for future work seem to be:

1. What factor(s) associated with long-standing, naturally occurring hyper PTH states, *depresses* the resorptive activity of the average osteoclast?

2. What increases the *number* of osteoclasts in hyper-PTH states?

3. A third question, concerning the mechanism by means of which *formation falls below resorption* in hyper PTH states, has been answered with acceptable statistical probability by the quantitative demonstration that tissue-level bone formation falls below the comparable resorptive parameter in these same cases (Hitt *et al.* 1969; Sarnsethsiri *et al.*, 1969; Villanueva *et al.*, 1969).

Preliminary attention to the second question has been given in practical terms by both Talmage (1967) and Young (1964), and in theoretical terms by Frost (1964, 1968).

References

Ashby, W. R.: An introduction to cybernetics. New York: John Wiley & Sons, Inc. 1963.

Berson, S. A., Yalow, R. S.: Parathyroid hormone in the plasma in adenomatous hyperparathyroidism, uremia and in bronchogenic carcinoma. Science **154**, 907 (1966).

Best, C. H., Taylor, N. B.: The physiological basis of medical practice, 7. ed. Baltimore: Williams & Wilkins Co. 1961.

Chalkley, H. W.: Area measurements in histology. J. nat. Cancer Inst. **4**, 47 (1943).

Dent, C. E.: Some problems of hyperparathyroidism. Brit. med. J. **1962 II**, 1419—1425.

Epker, B. N., Kelin, M., Frost, H. M.: Magnitude and location of cortical bone loss in human rib with aging. Clin. Orthop. **41**, 198—203 (1965).

Frost, H. M.: Measurement of bone formation in a 57 year old man by means of tetracycline labeling. Henry Ford Hosp. med. Bull. **10**, 267—285 (1962a).

— A model of endocrine control of bone remodeling. Henry Ford Hosp. med. Bull. **10**, 119—170 (1962b).

— Measurement of human bone formation by means of tetracycline labeling. Canad. J. Biochem. **41**, 31—42 (1963).

Frost, H. M.: Mathematical elements of lamellar bone remodeling. Springfield: Ch. C. Thomas 1964.
— Bone dynamics in osteoporosis and osteomalacia. Springfield: Ch. C. Thomas 1966.
— Tetracycline bone labeling in anatomy. Amer. J. Phys. Anthrop. **29**, 183—195 (1968).
— Villanueva, A. R., Ilnicki, L.: Tetracycline-based studies of bone remodeling in primary and secondary hyperparathyroid states in man. In: Parathyroid hormone and thyrocalcitonin, p. 123—136. New York: Excerpta Medica Found 1968.
— Wu, K.: Histological measurement of bone formation rates in unlabeled contemporary, archeological and paleontological compact bone. In: Miscellaneous papers in paleopathology. Technical Series No 7, Museum of Northern Arizona, p. 9—22 (1967).
Gaillard, P. J.: Parathyroid and bone in tissue culture. In: The parathyroids (eds. R. O. Greep and R. V. Talmage), p. 20—45. Springfield: Ch. C. Thomas 1961.
Harris, W. P., Heaney, R. P.: Skeletal renewal and metabolic bone disease. New Engl. J. Med. **280**, 193—202 (1969).
— — Jowsey, J., Haywood, E. A., Cockin, J.: The effect of growth hormone on skeletal metabolism in adult dogs. Abst. No 19 of Orthopeadic Res. Soc., 18 January, New York 1969.
Hitt, O., Jaworski, Z. F., Shimizu, A. G., Frost, H. M.: Tissue-level bone formation rates in renal dialysis patients, measured by means of tetracycline bone labeling. Manuscript in preparation (1969).
Hobdell, M. H.: Bone microradiography: some features of projection microradiographs. J. dent. Res. **42**, 132 (1967).
Hong, Y. C., Yen, P. K. J., Shaw, J. H.: Microscopic evaluation of the effects of some vital staining agents on growing bone in rabbits. Calc. Tiss. Res. **2**, 286—295 (1968).
Jaffe, H. L., Bodansky, A., Blair, J. E.: The influence of age and the duration of treatment on the production and repair of bone lesions in experimental hyperparathyroidism. J. exp. Med. **55**, 139—154 (1932).
Jett, S., Wu, K., Duncan, H., Frost, H. M.: Adrenal corticosteroid and salicylate actions on human and canine haversian bone formation and resorption. Clin. Orthop., **68**, 301—315 (1970).
— — Frost, H. M.: Tetracycline-based histological measurement of cortical endosteal bone formation in normal and osteoporotic rib. Henry Ford Hosp. med. J. **15**, 325—344 (1967).
— Ramser, J. R., Frost, H. M., Villanueva, A. R.: Bone turnover in osteogenesis imperfecta. Arch. Path. **81**, 112—116 (1966).
Johnson, L. C.: Morphologic analysis in pathology. In: Bone biodynamics (ed. H. M. Frost), p. 543—654. Boston: Little-Brown & Co. 1964.
Jowsey, J., Raisz, L. G.: Experimental osteoporosis and parathyroid activity. Endocrinology **82**, 384—396 (1968).
— Kelley, P. J., Riggs, L., Bianco, H. J., Scholz, D. A., Gershon-Cohn, J.: Quantitative microradiographic study of normal and osteoporotic bone. J. Bone Jt Surg. A **47**, 785—806 (1965).
Kaye, M., Silverman, M.: Calcium metabolism in chronic renal failure. J. Lab. clin. Med. **66**, 535—548 (1965).
Meuniere, M. P.: La lecture quantitative de biopsie osseuse. Rev. lyon. Med. **17**, 271—280 (1968).
— Personal communication (1969).
— Vauzelle, J. L., Vignon, G.: Etude anatomique des osteoses diffuse par lecture quantitative de la biopsie osseuse. Lyon méd. **14**, 1149—1169 (1968).
— Vignon, G., Vauzelle, J. L., Zech, P.: Etude histologique quantitative de la resorption osteoclastique dans les hyperparathyroides primitives et secondaires. Presse méd., in press (1969).
Needham, G. H.: The practical use of the microscope. Springfield: Ch. C. Thomas 1958.
Neuman, W. R., Dowse, C. M.: Possible fundamental action of parathyroid function in bone. In: The parathyroids (eds. R. O. Greep and R. V. Talmage), p. 310—326. Springfield: Ch. C. Thomas 1961.
Pirok, D. J., Ramser, J. R., Takahashi, H., Villanueva, A. R., Frost, H. M.: Normal histological, tetracycline and dynamic parameters in human mineralized bone sections. Henry Ford Hosp. med. Bull. **15**, 195—218 (1966).

Ramser, J. R., Villanueva, A. R., Frost, H. M.: Cortical bone dynamics in osteomalacia, measured by tetracycline bone labeling. Clin. Orthop. **49**, 89—102 (1966).

Rasmussen, H., Tenenhouse, A.: Thyrocalcitonin, osteoporosis and osteolysis. Amer. J. Med. **43**, 711—726 (1967).

Santoro, F., Frost, H. M.: Correlations of the transverse sizes of 6th and 11th ribs in normal and osteoporotic individuals. Henry Ford Hosp. med. J. **16**, 87—96 (1968).

Sarnsethsiri, R., Jaworski, S. F., Shimizu, A. G., Frost, H. M.: The appositional rate of new bone formation, and osteoid seam thickness, in patients undergoing renal dialysis. Arch. Path. 88, 49—53 (1969).

Saxen, L.: Effect of tetracycline on osteogenesis *in vitro*. J. exp. Zool. **162**, 269—294 (1966).

Sedlin, E. D.: Uses of bone as a model system in the study of aging. In: Bone biodynamics (ed. H. M. Frost), p. 655—668. Boston: Little-Brown & Co. 1964a.

— The ratio of cortical area to total cross-section area in rib diaphysis: a quantitative index of osteoporosis. Clin. Orthop. **36**, 161—168 (1964b).

Takahashi, H., Frost, H. M.: Age and sex related changes in the amount of cortex in normal human ribs. Acta orthop. scand. **37**, 133 (1966).

Talmage, R. V.: A study of the effect of parathyroid hormone on bone remodeling and on calcium homeostasis. Clin. Orthop. **54**, 163—173 (1967).

Villanueva, A. R., Jaworski, Z. F., Sarnsethsiri, P., Shimizu, A. G., Frost, H. M.: Tissue-level bone resorption rates in renal dialysis patients and in primary hyperparathyroidism, measured by tetracycline bone labeling. Manuscript in preparation (1969).

Weibel, E. R.: Principles and methods for the morphometric study of the lung and other organs. Lab. Invest. **12**, 131—155 (1963).

Wu, K., Jett, S., Frost, H. M.: Bone resorption rates in physiological, senile and postmenopausal osteoporosis. J. Lab. clin. Med. **69**, 810—818 (1967).

— Schubeck, K. E., Frost, H. M., Villanueva, A. R.: Haversian bone formation rates determined by a new method in human diabetes mellitus, osteogenesis imperfecta and osteoporosis, and in a Mastodon. Calc. Tiss. Res. in press (1970).

Young, R. W.: Specialization of bone cells. In: Bone biodynamics (ed. H. M. Frost), p. 117—139. Boston: Little-Brown & Co. 1964.

Zeiss, C.: Integrating eyepiece leaflet 40—195e (Oberkochen) (1960).