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TETRACYCLINE-BASED MEASUREMENT OF BONE DYNAMICS IN RIB OF A GIRL DYING OF GAUCHER'S DISEASE***

JOHN E. PASKO* AND HAROLD M. FROST, M.D.**

INTRODUCTION

We have studied the bone dynamics in a child with Gaucher's disease by means of *in vivo* tetracycline labeling and mathematical analysis of histological measurements of undecalcified rib sections, using Frost's method.^{8,14,17,18,22,23,27,30,32} Previous experience with this technique suggested that one of three situations might be found in this child: (i) there could be changes in bone structure or in its dynamics which were direct consequences of the disease, i.e., *primary changes*; (ii) there could be changes which were secondary to others which themselves could be primary or secondary, i.e., *secondary changes*; (iii) or there could be no measurable abnormality.

Primary changes have been found by this method of study in osteoporosis,^{29,30} osteomalacia,¹⁸ acromegaly,²⁰ osteogenesis imperfecta,^{11,21} pseudohypoparathyroidism,¹ Cushing's syndrome,¹⁴ and rheumatoid arthritis.¹⁹

We believe the findings in this case are secondary. They are interesting for they showed something which has not been shown before: the mechanism of the marrow cavity expansion in a disease in which the medullary soft tissue volume enlarges. The data also suggested a way to distinguish between the effects on cell behavior of systemic and local regulatory factors.

The "envelope" concept that will be used in the subsequent text^{9,10} means that all Haversian canal surfaces or walls are a collective, functionally distinct surface on which Haversian bone remodeling occurs and to which it is confined. This surface envelopes a volume of space in which lie the soft tissue contents of Haversian canals. The cortical-endosteal envelope is the endosteal surface of the compacta, it contains the marrow cavity plus its soft tissues, and cortical endosteal remodeling is confined to this surface and is functionally distinct from Haversian remodeling. From the foregoing, the meaning of a third, periosteal, envelope is clear. These envelopes have been shown to possess some functional independence by Sedlin,²³ Smith and Walker²⁴ and Takahashi and Frost.²⁶

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MATERIALS

At the time of her death the patient (HFHORL* code: B_n), a white female of Greek ancestry, was 42 months old. The diagnosis of Gaucher's disease had been made at the age of 11 months by iliac marrow aspiration. Her course from that time was one of progressively increasing illness with marked hepato-splenomegaly, lymphadenopathy, bone involvement, ascites and anemia. She received multiple medications (but not in large doses or for prolonged periods of time) and many blood transfusions, had a splenectomy done at age 18 months, and was continuously febrile and stuporous during the last six weeks of life. She was 35 inches tall. She weighed 38 lbs. at death. Much of this weight was due to visceromegaly, for the liver weighed over 3kg. at autopsy while her extremities were emaciated.

During her last month of life typical laboratory values included: a serum acid phosphatase of 8.4 - 16.6 KA. units; serum alkaline phosphatase of 12.7 Bodansky units. Platelets 40,000 - 120,000/mm³; hemoglobin 5.6 - 7.8gm%; reticulocytes 14.4%, sickle cell preparation was negative; hemoglobin electrophoresis showed hemoglobin A only. Serum electrophoresis showed 2.9gm of gamma globulin. The urine showed albumin and RBC.

X-rays showed progressive cortical thinning, osteoporosis, marrow cavity expansion and patchy demineralization of her axial and proximal appendicular skeleton. She sustained three pathological fractures in her last year of life which healed at a normal rate. See figures (1,2).



Figure 1

AP x-ray of the pelvis and hips. The deformity at the proximal end of the left femur is the consequence of one of her fractures.

*Henry Ford Hospital Orthopaedic Research Laboratory.

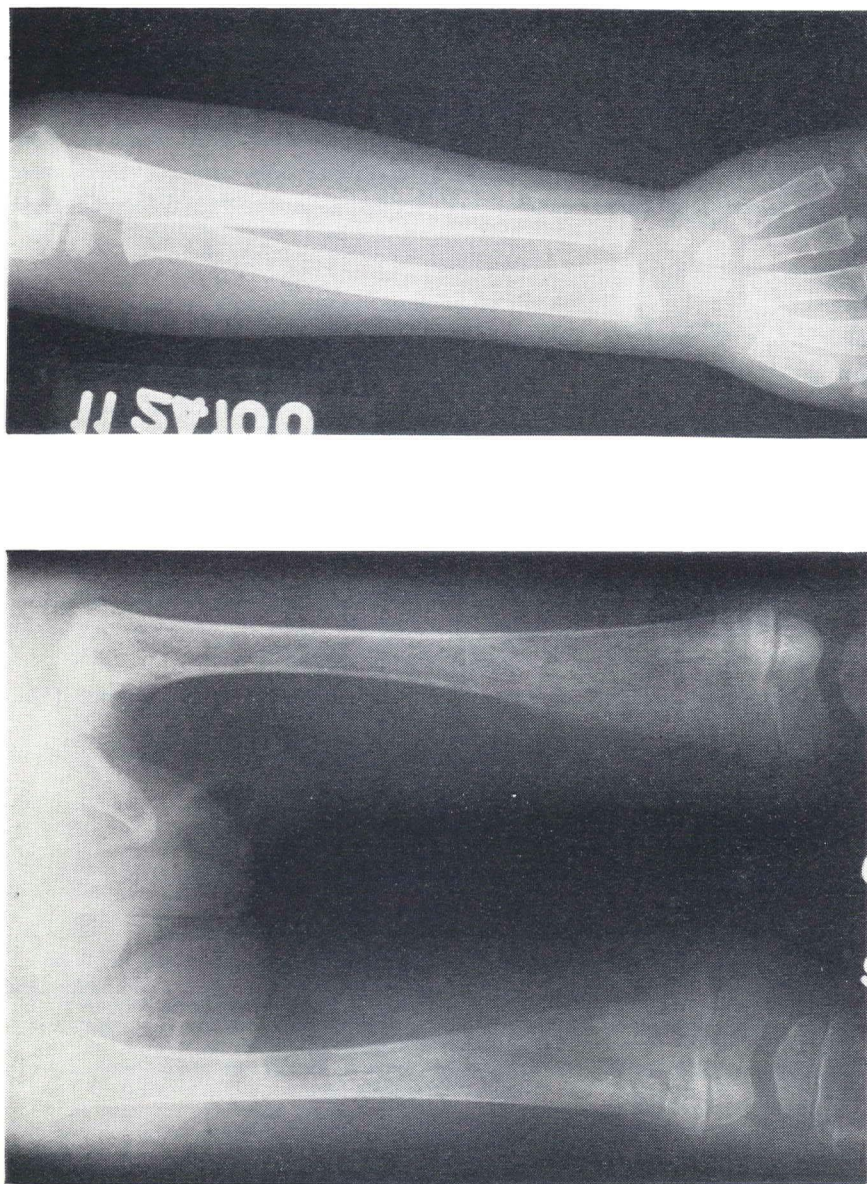


Figure 2

Left: AP x-ray of the right (unfractured) femur, showing the osteoporosis and marrow cavity expansion. Note the thinness of the cortices relative to the external diameter of the bone.
Right: AP x-ray of the forearm and hand, showing marked cortical thinning due to an enlarged marrow cavity in the radius, ulna and metacarpals. There is also some patchy demineralization.

She was given a double tetracycline bone label six weeks before death. The label consisted of two three day courses of demethylchlortetracycline* 20mg/kg/day with a 15 day interval between. At the time of autopsy, the middle third of the 6th rib was removed for analysis.** Extensive studies of normal bone at this sampling site provide the basis for comparison.

METHODS

Sections: Fresh, undecalcified, hydrated, 70 micron thick cross sections were made of the rib. Some were stained with the tetrachrome bone stain[±], others were unstained. They were dehydrated, cleared and mounted in Harleco Synthetic resin (H.S.R.),^{6,7,28}

Measurements: See figure (3). A series of basic measurements were made under the light microscope by methods described previously.^{8,14,19,30} These included: cortical cross section area (A_c); total cross section area (A_t); number of osteoid seams (S_r) and resorption spaces (S_r); number of tetracycline labels; appositional rate (M); endosteal circumference ($^{\circ}S$); mean wall thickness (mwt).

Computed Data: The following values were computed⁺⁺ from these data to obtain measures of the bone *tissue* and *cell population* dynamics:

- a) Bone forming and resorbing centers per mm² of cortical cross section area (hA_r , hA_r).
- b) Bone forming and resorbing centers per mm endosteal circumference ($^{\circ}A_r$, $^{\circ}A_r$).
- c) Radial closure rate; on each envelope (hM_r , $^{\circ}M_r$).
- d) Bone formation rate; on each envelope (hV_r , $^{\circ}V_r$).
- e) Bone formation period; for each envelope ($^h\sigma_r$, $^{\circ}\sigma_r$).
- f) Mesenchymal cell activation function; on each envelope ($^h\mu$, $^{\circ}\mu$). These data are listed in Tables I and II.

*Declomycin, Lederle.

**We are greatly indebted to Drs. R. Monto, D. C. Mitchell, R. Horn and G. Fine for the opportunity to study this case.

+Available as the "Osteochrome", from Harleco, Philadelphia, Pa.

++Using equations in Chapters V and VI, Frost (1964).

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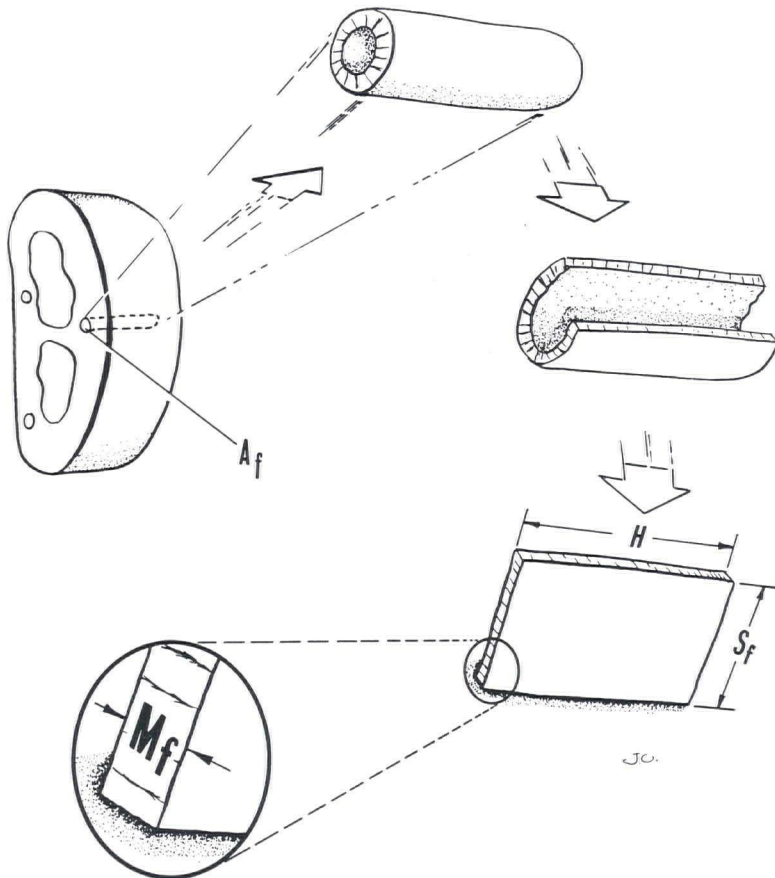


Figure 3

This figure explains diagrammatically the physical basis of Frost's quantitative histological analysis of undecalcified tetracycline labeled bone sections. A 1mm thick slice of rib is shown in exploded three quarter view. (H) is the height of the osteoid "cylinder", and is unity since the section of rib containing it is one mm thick. The area of the wall of this osteoid cylinder is its circumference times its height (i.e., $S_r \times H$). Since H always has the numerical value of one, it is omitted from calculations. When the surface area of the seam is multiplied by the thickness added to it per year (M_r), the result is the rate of bone formation in this single center. When this value averaged for all centers is multiplied by their number (A_r), the result is the total amount of bone being made in the rib. If divided by the amount of bone in the rib slice A_o , the result is bone formation per mm^3 of cortex per year, or hV_r .

A similar analysis is made of cortical endosteal bone formation.

Table I
Bone-Histological Data in Gaucher's Disease

Parameter	Units	Patient	Typical Normal Value Age 46 months	Symbols in text
Cortical cross section area per section	mm ²	11.2	11	A _c
Total cross section area per section	mm ²	20.4	19.8	A _t
Total cortical endosteal circumference per section	mm	16.5	9.5	^e S
Haversian seams, total number	—	35	140	—
Cortical endosteal seams, total number	—	18	8	—
Haversian resorption centers, total number	—	59	68	—
Cortical endosteal resorption centers, total number	—	26	—	—
Per cent seams labeled	—	100	90 - 100	—
Thickness osteoid seams	mm	.0095	.0076 ± .07	—
C/T ratio of Sedlin	—	.55	.61 ± .05	C/T
Parabolic index of Epker	—	.247	.24 ± .01	k

Raw data for this case, with age comparable norms where available and pertinent.

Table II
Histological and Histodynamic Data in 6th Rib of a Girl with Gaucher's Disease

Parameter	Haversian			Endosteal		
	Patient	Normal (S.D.)	Symbol	Patient	Normal (S.D.)	Symbol
Osteoid Seams number per mm	1.6	4.0 ± 1.0	^h A _f	.54	.40 ± .06	^e A _f
Resorption spaces number per mm	2.6	3.0 ± 1.5	^h A _r	.79	—	^e A _r
Mean seam circumference mm	.39	.30 ± .05	^h S _f	.32	.50 ± .1	^e S _f
Radial closure rate, mm/yr	.35	.52 ± .06	^h M _f	.65	.56 ± .08	^e M _f
Mean wall thickness mm	.068	.068 ± .007	^h mwt	.075	.30 ± .03	^e mwt
Bone formation rate mm ³ /yr	.21	.63 ± .2	^h V _f	.11	.08 ± .04	^e V _f
Formation period years	.20	.13 ± .015	^h σ _f	.12	.5 ± .07	^e σ _f
Mesenchymal cell activation frequency	8.0	30.0 ± 10	^h μ _f	4.6	.80 ± .3	^e μ _f

Computed data for this case for each envelope, with age comparable norms for comparison. The formation period is the length of time taken to make one of the bone moieties shown in figure (5). The activation frequency is the number of new moieties whose formation begins, per year per unit amount of bone. The Haversian bone formation rate is the annual amount of bone made per mm³ of compacta. The endosteal rate is the annual amount of bone made per mm² of cortical endosteal surface.

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RESULTS

Table I lists the raw data. Table II presents the computed data and compares them to age comparable norms.^{12,17}

With respect to normal, the major findings in this child were:

1) *Haversian Envelope*: The bone formation rate was a third of normal. The paucity of osteons in the cortex prove that this rate had been subnormal for a long time; see figure (5). While osteons took twice the normal completion time (σ)^(a), only a sixth of the normal number of new osteons was begun per year (μ)^(a). All systems with seams were labeled with tetracycline, which is normal in children. Osteon wall thickness was normal at .068mm. The osteoid seams were .0095mm thick which is 25% greater than, but within one S.D. of, normal.³² The mean seam circumference was increased 30%, which was not significant. The number of active bone forming centers was a fifth of normal,^(a) while the number of resorption spaces was half of normal. See figure (4.)

2) *Endosteal Envelope*: The bone formation rate was essentially normal. The bone formation period was 24% of normal,^(a) mostly because the mean wall thickness was decreased to less than 20% of normal^(a) so that it took less time to deposit this thinner layer of new bone. The radial closure rate at .65mm/yr. was within one S.D. of normal. More than three times the normal number of new bone forming centers were being created per year per mm² of cortical endosteal surface.^(a) The number of osteoid seams was normal but their circumference was 70% of normal. Norms have not yet been established for the number of resorption centers on the cortical endosteal surface.

3) *Cross section areas*: The cortical cross section area and the total cross section areas were within one S.D. of normal for her height.²⁷ Her C/T ratio of 0.55 was one standard deviation smaller than normal,²³ so her marrow cavity was larger than it should have been for the amount of compacta that was present.*

4) *Histological structure and polarized light studies*: No major qualitative abnormalities were noted in the microscopic architecture of the bone, in the lamellar widths, nor in the contour and distribution within the compacta of the osteons. Osteoclasts, osteoblasts and mast cells were seen in numbers that appeared visually to be proportional to the turnover figures. These relatively crude observations would only detect large abnormalities.

5) *Staining patterns*: The permeability of the bone to basic fuchsin was normal, implying that there was no major disturbance in the second stage of mineralization nor in osteocyte metabolism.^{7,13,16,31} The permeability and tinctorial patterns with the tetra-

^(a)Significant at the .05 level.

*Note that the variance found by the authors quoted was *interperson* variance and reflects the fact that the bone samples they studied were taken from people of varying height, weight and body size. When the variance is studied among subjects of similar height, weight and age, it is found that the coefficients of variation in these parameters are typically .03. Therefore the numerically small abnormality in this child is probably more meaningful than it seems to be at first, although published figures do not exist to prove this inference.

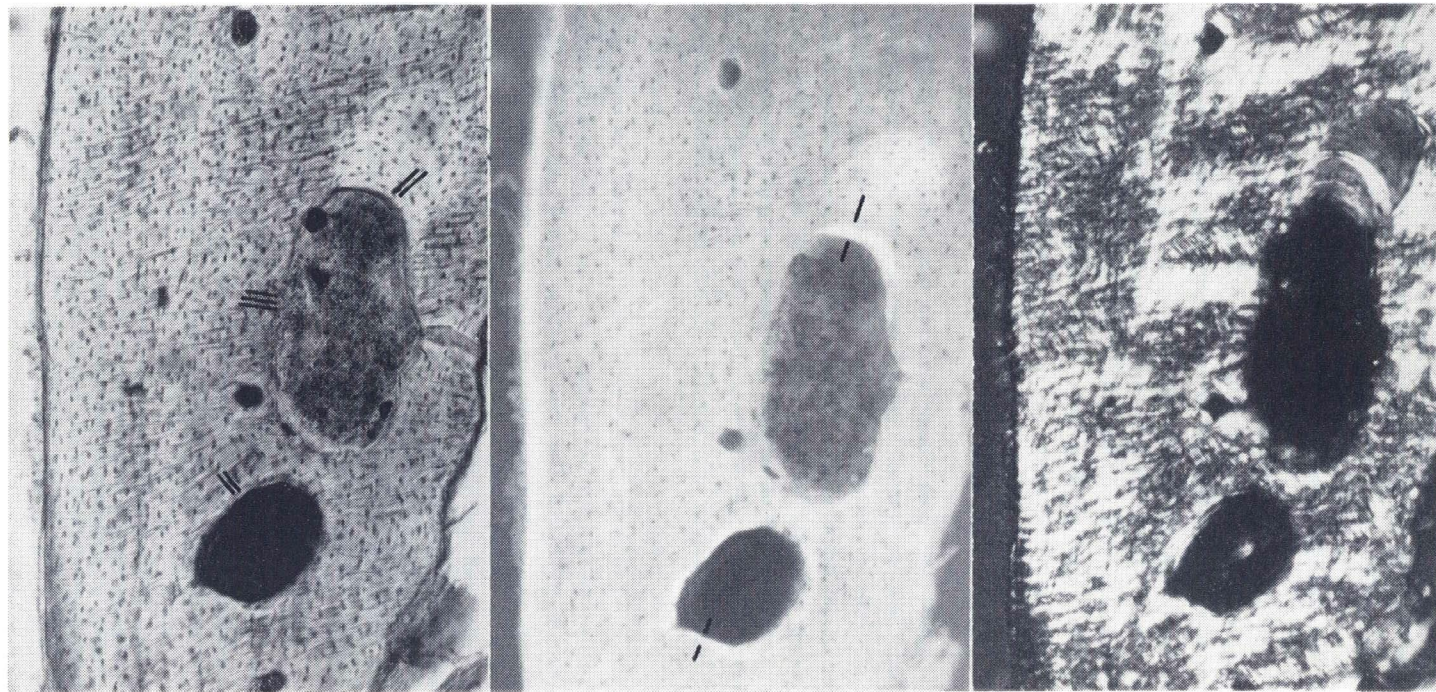


Figure 4

Left: Undecalcified cross section of the rib, showing the periosteal surface on the left, a resorption space (triple tick marks) and osteoid seam lining an actively forming new Haversian system (double tick marks), and the endosteal surface on the lower right.

Middle: Fluorescence microscopy of the same field. The tick marks identify tetracycline labels at the periphery of the osteoid seams.

Right: Same field, between crossed polars. The appearance is normal for a young child.

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chrome sain were normal,²⁸ meaning no major abnormality existed in chemical composition of the organic matrix nor in the osteoid seams which would be revealed by these means. These qualitative observations do not preclude all such abnormalities.

DISCUSSION

1) *Haversian Envelope Dynamics*: A decreased bone formation rate and "birth rate", (see figures 5) plus a prolonged osteon formation period and increased ratio of resorption cavities to osteoid seams (i.e., 1.7 as opposed to normal in children of 0.8) were also found in patients treated with exogenous adrenal cortical steroids.¹⁴ We have found but not reported similar changes in many other patients with severe, protracted and varied illnesses, so these findings are not unique to Gaucher's disease. In this girl these abnormalities could reflect endogenous adrenal cortical steroid production, which could have been increased in response to the physiological stress of severe illness, multiple therapeutic procedures and fractures. Perhaps impaired metabolism of steroids in her enormous liver was also a factor. It is not unusual that her osteons were normal in size (index: mean wall thickness) for this quantity seldom undergoes a major change in disease.^{4,15,30}

2) *Endosteal Envelope Dynamics and the Envelope Concept*: The supernormal creation of new bone forming centers coupled with the subnormal mean wall thickness, seam circumference and bone formation period, are changes which were absent, or actually the opposite of those found, on the Haversian envelope, which shows that cell behavior on the two envelopes can vary independently.* Since the same course of blood

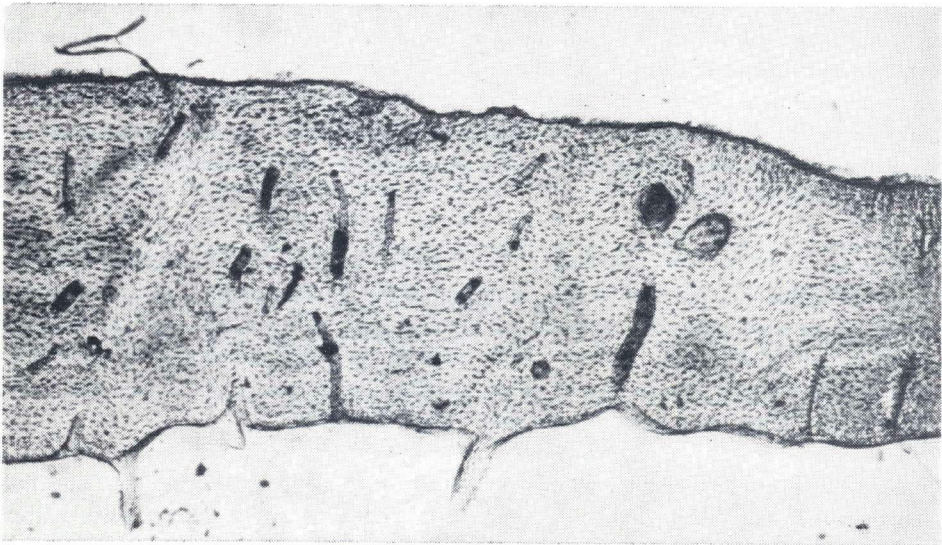


Figure 5

Undecalcified cross section of pleural cortex of the rib. There are only two secondary osteons here, proving that Haversian turnover of the compacta has been slow for a long time. This proves that the low bone formation rate and the decreased number of new osteons being created per unit time found at the time of labeling were representative of the usual rates.

*The idea that this might happen is not original but this demonstration that it does is.

peruses all cells, systemic factors probably do not cause such interenvelope behavioral differences. Rather either (i) factors which are local with respect to the cells are involved, or (ii) the cells on each envelope are dissimilar in their functional potentials.³⁰ Since there are convincing reasons for discounting (ii),⁵ a local factor(s) is implied.

3) "*Resolution*" of Systemic and Local Regulatory Factors: On the basis of these and other observations, we propose this hypothesis: Dynamic *abnormalities* in cell behavior on the Haversian envelope usually reflect blood-borne systemic regulation by factors, such as hormones, nutrition and vitamin abnormalities and defective absorption of minerals. Besides being influenced by systemic factors, cell behavior on the periosteal and endosteal envelopes reflects the added action of local factors in the bone, for example surface bioelectric potentials³ or polarized ion pumping due to mechanical strain.²⁵ If this were true, then to a first approximation one could resolve between and measure the action of systemic and local regulation on cell behavior on these two envelopes, by comparing their differences with their absolute values. This capability would be a great help in interpreting studies of bone physiology, disease and/or its treatment.

4) *Marrow Cavity Expansion*: An enlarged marrow cavity existed in this girl, shown by measurement of her rib cross sections, and by the presence of osteoporosis on her x-rays (see figures (1,2)). The probable mechanism of this expansion is interesting. To understand it, two basic properties of remodeling must be reviewed.

First, on any given bony envelope and in steady state* situations (i.e., states lasting in excess of 6 months), changes in the bone resorption and formation rates seem to parallel each other, so that if one is increased so is the other, and conversely. The magnitude of these changes may be unequal, which allows gain or loss of bone to occur.⁹ Since the girl's endosteal formation rate was increased, it may be assumed her resorption rate was also increased. These increases were due to heightened progenitor cell activity (index: μ).

Second, lamellar bone remodeling** occurs in multicellular, functionally discrete "packets" or BMU in which an initial resorptive phase is followed by a formative phase. On normal cortical endosteal surfaces 1.8X more bone is resorbed than is made per BMU,³³ which makes the marrow cavity enlarge as long as endosteal remodeling occurs.*** In this girl the amount of bone made by the formative phase was subnormal (index: mwt), implying a greater resorptive excess per BMU.

Thus there are two distinct ways to increase cortical endosteal bone loss, and thus to enlarge the marrow cavity to accommodate an increasingly voluminous marrow tissue. The first is by enlarging the resorptive excess per BMU, the second by accelerating

*This does *not* mean equilibrium; see Ashby.²

**Remodeling is used in the sense of turnover without significant change in the amount or shape of the bone. It is to be distinguished from modeling, which is confined to periosteal and endosteal surfaces and which can alter the amount of bone and its geometry.

***And possibly is correct in implying that osteoporosis might be prevented by depressing endosteal remodeling early in adult life.

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the creation of new remodeling packets.† Both mechanisms acted in this child. This differs from the previous view that such enlargement follows resorption which occurs independently of bone formation and independently of bone progenitor cell* activity.

SUMMARY

Bone dynamics in a child, who died of Gaucher's disease, were studied by doing a quantitative histological analysis of mineralized rib sections labeled with tetracycline before death. Bone formation was decreased on the Haversian, but increased on the endosteal envelopes. Haversian systems were normal in size but cortical-endosteal bone moieties were subnormal. Creation of new bone forming centers was subnormal on the Haversian but supernormal on the endosteal envelope. The marrow cavity was enlarged, apparently because (i) too little bone was made per cortical-endosteal remodeling "packet", and (ii) too many such packets were created. These findings are not unique to Gaucher's disease; they are interpreted as the consequences of (i) a protracted severe illness, (ii) coupled with an enlarging volume of marrow tissue.

It is suggested that measurement and comparison of cell behavior on Haversian and cortical endosteal envelopes may permit the effects of systemic and local regulation on bone cells to be "resolved" and measured.

†Thus if 10 remodeling packets each lose $.020\text{mm}^3$ of bone per year (total: $.20\text{mm}^3$), then 20 packets will lose twice as much (total: $.40\text{mm}^3$), and 30 three times as much (total: $.60\text{mm}^3$).

*Bone progenitor cell, osteoprogenitor cell and mesenchymal cell are taken to be synonymous in this text, and are functionally oriented definitions rather than structural or biochemical definitions.

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