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BONE FORMATION IN HUMAN OSTEOGENESIS IMPERFECTA, MEASURED BY TETRACYCLINE BONE LABELING

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The disabling and occasionally fatal skeletal disease known as osteogenesis imperfecta (OGIM) (synonyms: brittle bones; osteopsathyrosis; Loebstein's disease) manifests itself in part by an insufficient quantity of bone in the skeleton, associated with a mechanical fragility which may lead to numerous and repeated fractures, some following minimal trauma and some occurring "spontaneously". As a consequence of these fractures plus an inability to model deformed bones in the normal way, serious skeletal deformities frequently develop. (Aegerter & Kirkpatrick 1968, Caniggia et al. 1958, Rubin 1964). This condition has been attributed to an inability of osteoblasts to manufacture bone as fast as the growing fetal and infant skeleton required it (Aegerter & Kirkpatrick 1968, Caniggia et al. 1958). Lacking direct evidence to the contrary, this straightforward hypothesis has gained wide acceptance as the probable explanation for the skeletal features of OGIM. The advent of tetracycline bone labeling (Milch et al. 1958, Lee 1965) and the development of techniques which use it to measure the dynamics of bone formation directly in bone tissue now make it possible to study bone formation dynamics with greater certainty and accuracy in humans afflicted with metabolic bone diseases. This article summarizes this laboratory's experience with measurements of bone formation rates in humans with OGIM, as revealed in bone biopsies by the tetracycline bone labeling phenomenon.

MATERIALS

This study includes 11 patients (7 males and 4 females) with obvious OGIM who contributed 12 biopsies of 12 bones. Three of these patients were reported previous-

Table 1. Osteogenesis imperfecta subjects

Code	Age	Sex	Clinical history
N_j	3	♀	Many spontaneous fractures in lower extremities. Generalized osteoporosis. No bone disease in family.
I_s	13	♂	Three spontaneous vertebral collapses; perfectly healthy until this. Generalized osteoporosis; blue sclerae. No history of bone disease in family.
O_j	13	♂	Several vertebral collapses; deformed (R) femur; multiple past fractures of long bones. Family history of imperfecta.
C_d	15	♂	Bilateral fractures femurs at birth; apparently normal until age 10, then 8 fractures of long bones. Osteoporosis, dentinogenesis imperfecta; mother has blue sclerae.
C_q	24	♂	20 fractures by age 13; none since then. Osteoporosis. No history of imperfecta in family.
I_t	28	♂	9 fractures from age 9 to 20. Bone biopsy diagnosed as idiopathic osteoporosis at another hospital. Multiple compression fractures with marked generalized osteoporosis.
A_e	30	♀	60 fractures until age 12. Family history of imperfecta.
A_f	51	♀	Multiple fractures. Severe deformities lower extremities and kyphoscoliosis. Family history of osteogenesis imperfecta, blue sclerae, otosclerosis.
A_g	70	♀	Multiple vertebral collapses; multiple long bone fractures and blue sclerae. Spinal osteoporosis; brother and sister have osteogenesis imperfecta.
B_u	4½	♂	Multiple past fractures, upper & lower; severe deformities. No family history.
O_e	11	♂	Multiple past fractures. Severe deformities of femurs and tibias. No family history.

The basic clinical data for each case in this study. The code letters identify a given case, and remain identical for that case in all publications from this laboratory.

ly and eight were not. Their ages ranged from 3 to 70 years. Three of them were submitted through the generosity of J. King, M.D. (Toronto, Canada), M. Castle, M.D. (Detroit, U.S A.), and P. Meuniere, M.D. (Lyons, France). Table 1 summarizes the case material. In all of these patients, numerous determinations of serum calcium, inorganic phosphate, and alkaline phosphatase, and of 24 hour urine calcium ex-

cretion yielded normal values. None of the patients has a known associated renal, endocrine, nutritional, or hematological disorder. All arose from Caucasian stock.

METHODS

(1) *Labels and biopsies:* All patients received one or two orally administered tetracycline labels, and a bone biopsy was done, more than one but less than three weeks after completion of the last marker, of the 11th rib in nine patients and in two other patients of one or more of the long bones of the lower extremities, samples of which were removed at the time of elective operation (usually the Sofield "shish-ke-bab") for correction of bony deformities. Table 2 lists the bone(s) examined in each case.

(2) *Sections and stains:* Undecalcified, accurately oriented complete cross-sections of the bone samples were ground by hand under running water on the day of receipt of the specimen and stained as described elsewhere (Frost 1969). At least 3 sections per case were prepared (except Oe, whose two plastic-embedded sections were submitted already prepared by Dr. King) and mounted in Harleco Synthetic Resin for permanent reference.

(3) *Measurements:* By means of blue-light fluorescence microscopy, the tissue-level *bone formation rate* of new haversian bone made (as seen in thin cross-sections) per mm^2 of pre-existing compacta, on an annual basis, was measured directly, as described elsewhere (Frost 1969). The mean distance between the middles of the two tissue time markers for bone formation also was measured in each section of each case. This distance, divided by the labeling interval, equals the *appositional rate* of new bone formation and reflects bone formation at the osteoblast level (Frost 1969, Villanueva et al. 1966). See Figure 1.

Table 2 presents the above data, as well as age-comparable normal data, both in absolute units and as percentages of age-comparable norms, since especially in children the speed of bone formation varies considerably as a function of age.

RESULTS

(1) *Tissue-level bone formation rate:* This rate averaged i.e. 309 per cent ± 70 per cent (S. E. = 23 per cent) of normal for the group of 9 rib biopsies, which differs significantly from normal (which in comparable terms averages 100 ± 30 per cent; S. E. = 2 per cent) by inspection ($p < .05$).

Only one of these patients (I_s) demonstrated a really subnormal rate, yet his depression still fell within one S. D. of his comparable norm.

While normal standards for the human tibia and femur do not yet exist, other studies indicate that in man their formation rates average 10 to 50 per cent of the values for the 6th and/or 11th ribs (Frost 1969). On this basis the value of this rate fell within the normal range in case B and exceeded normal in both bones examined in case O_s.

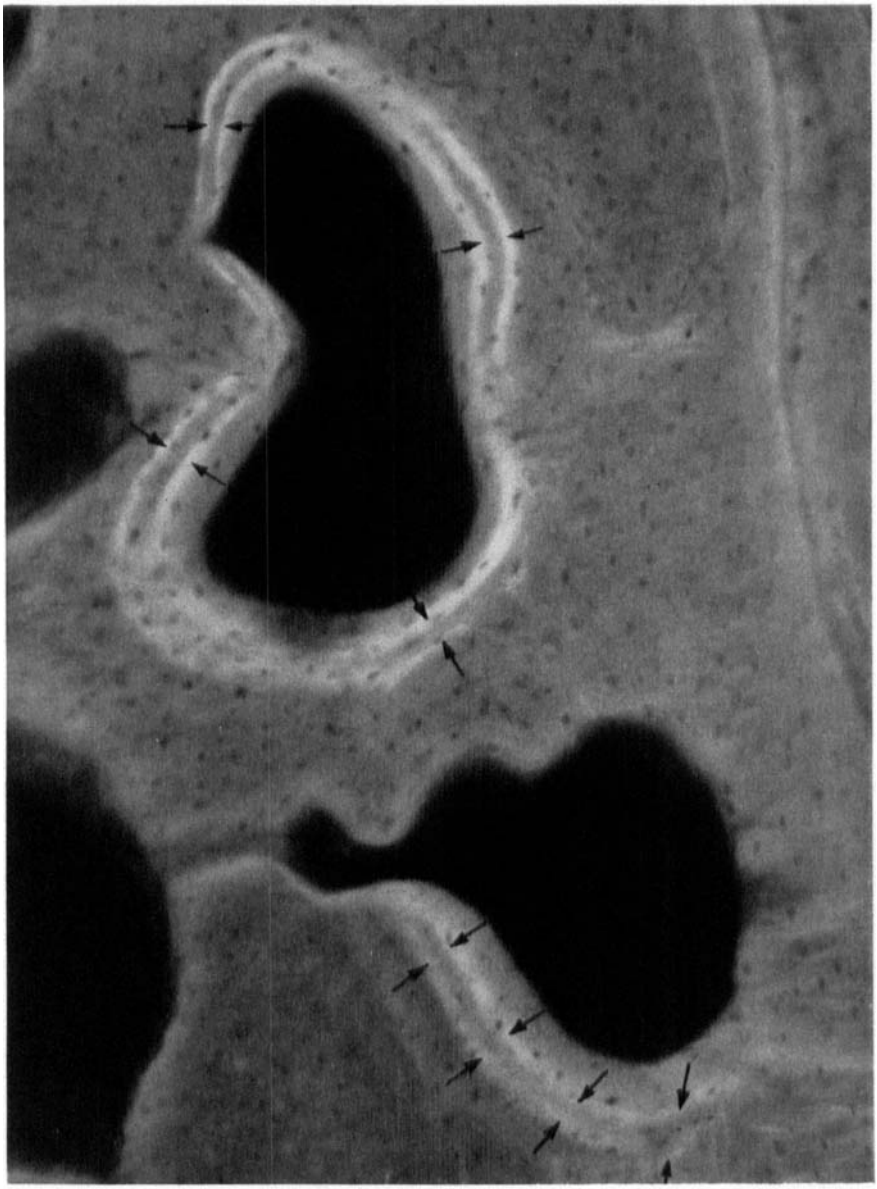

Figure 1. A photomicrograph of a 3–10–6 (double) tetracycline label in a child with osteogenesis imperfecta. Undecalcified cross-section 11th rib biopsy, 320X, blue-light fluorescence. We made four measurements of each labeled system at four intervals equally spaced around the perimeter of each; the mean of all these measurements divided by the time between the middle of each marker, represents the appositional rate of the text.

Table 2. Histodynamic data in 11 cases of osteogenesis imperfecta

Code letter	Bone biopsied	OGIM (M) (microns per day)	Age comparable normal (M) 11th rib (microns/ day)	Per cent of normal (M)	OGIM (Vf) (mm^2/ mm^2/ yr)	Age comparable normal (Vf) 11th rib (mm^2/ mm^2/yr)	Per cent of normal (Vf)
N_j	11th rib	1.1	1.4 ±0.46	79	0.37	0.38 ±0.24	97
I_s	11th rib	1.4	1.3 ±0.43	108	0.096	0.21 ±0.15	46
O_i	11th rib	1.2	1.3 ±0.43	92	0.76	0.21 ±0.15	362
C_d	11th rib	1.4	1.3 ±0.43	108	0.29	0.21 ±0.15	138
C_q	11th rib	0.94	1.2 ±0.60	78	0.21	0.067±0.037	313
I_t	11th rib	1.1	1.2 ±0.60	92	0.22	0.067±0.037	328
A_e	11th rib	0.53	1.1 ±0.36	48	0.14	0.018±0.006	778
A_f	11th rib	0.63	0.90±0.30	70	0.097	0.036±0.012	269
A_g	11th rib	0.77	0.72±0.20	107	0.20	0.044±0.015	455
B_u	Femur	0.83	1.4 ±0.46	59	0.17	0.38 ±0.24	–
O_e	Femur	2.00	1.3 ±0.43	154	0.39	0.30 ±0.10	–
	Tibia	1.70	1.3 ±0.43	131	0.49	0.30 ±0.10	–

The data and the bones examined for each case. M: The appositional rate, expressed in microns per day. V : The bone formation rate, expressed as mm^2 of new bone made per mm^2 of pre-existing compacta on an annual basis. The Table also lists the data for each case as the per cent of the age-comparable norm of the 11th rib biopsy site. No adequate standard of normal bone formation rates exists for the femur and tibia in man, but these rates usually range from 10 per cent to 50 per cent of the value in ribs.

In nonparametric terms, the probability that tissue-level bone formation in OGIM proceeds at subnormal speed, and that these data merely reflect a sampling problem in which 11 out of 12 bone samples were nonrepresentative, falls below .001. Accordingly the elevation of the group carries high statistical significance.

(2) *Appositional rate:* This averaged 88 to 20 per cent of normal for the group of nine rib biopsies. Unlike the tissue-level formation rate, which may adopt characteristic but different values from bone to bone in the same skeleton, the appositional rate tends strongly towards uniform values throughout any given skeleton (Frost 1969). For this reason one may properly compare the values found in the tibia and femurs to normal standards for ribs. The appositional rate differed by more than one S. D. from the normal mean in only two of the 11 cases (cases A_e and B_u), whose values still fell less than 2 S. D. below their comparable norms. The mean for all 11 patients equalled

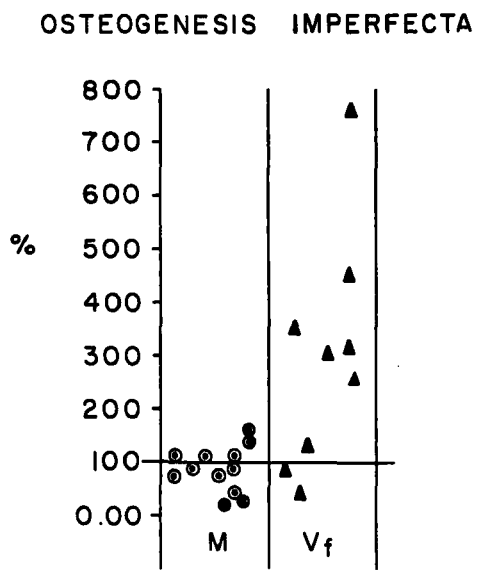

Figure 2. The values for the appositional rate and bone formation rate of each case were normalized by expressing each as the per cent of his or her age-comparable norm. M was normalized for all 11 cases and 12 bones, but the formation rates were normalized only for the 9 rib biopsies since normal values of this parameter differ in characteristic ways in different bones of the skeleton.

91 ± 31 per cent (S. E. = 6.3) of normal. In comparable terms normal equals 100 ± 30 per cent; S. E. = 2 per cent.

(3) Figure 2 presents the above data in scatter plot form, after normalizing each case to his or her age-comparable norm (Frost 1969).

DISCUSSION

(1) *Cellular level index:* We define cellular level bone formation as the average amount of new bone made by the average osteoblast in unit time. Here the appositional rate serves as an index of this activity. The appositional rate in these imperfecta patients fell within one standard deviation of normal in all but two of the 11 cases and 12 bones examined, and lay above the mean normal value in five, and below the mean normal level in six of the 11 cases. Since much greater depressions in this parameter accompany perfectly adequate accumulation of skeletal mass during growth in vitamin D resistant rickets (Villanueva et al. 1966), the small depression found here does not

represent a dynamic abnormality which could account for the deficiency in bone mass which characterizes OGIM. Note that this study did not define in any way the *quality* of the bone produced; it measured only the *rate* of its production.

(2) *Tissue level bone formation:* Tissue-level bone formation represents that made by the average osteoblast multiplied by the number of these cells in a unit amount (here one cubic millimeter) of bone. At this level of biological organization, the group's bone formation rate averaged 309 per cent *supernormal,* and only one case had a subnormal rate. Thus at this level, too, no justification appeared to explain their subnormal skeletal masses as an inability to make new bone as fast, or in such amounts, as body growth generally requires. This statement even applies to the one case with a subnormal bone formation rate, for again in vitamin D resistant rickets much greater depressions in this rate have accompanied normal, if indeed not supernormal, accumulations of skeletal mass (Frost 1969).

Interestingly, Lee (1965) has reported very similer cell-level and tissue-leve lfindings in two young children with osteogenesis imperfecta whom he examined.

Accordingly, and contrary to former belief, patients with osteogenesis imperfecta apparently can and do make new bone in adequate amounts at both the cellular and tissue-levels. By the process of elimination this suggests that an inability to *retain* new bony tissue constitutes the cause of the subnormal bone mass which characterizes this disease, i.e., too much bone resorption exists, an excess which furthermore manifests itself primarily on the periosteal, cortical-endosteal, and trabecular surfaces, as reflected by the thin cortices and sparse amounts of spongiosa but relatively normal cortical porosities which characterize the skeletons of imperfecta patients. A preliminary analysis of the patterns of anatomical distribution of this defect suggested (but did not prove) that periosteal and endosteal bone cells may respond to abnormal "signals" in their local microenvironment, signals which ultimately arise from mechanical forces acting on a skeleton whose structural material incorporates some qualitative abnormality which causes it to generate the abnormal "signals" (Ramser et al. 1966).

(3) The present study illuminates only one aspect of the manifold features of OGIM, that of the deficient accumulation of total bone mass. It does not shed any obvious light upon the abnormal staining properties of decalcified sections of osteogenesis imperfecta bone,

(properties which imply a qualitative defect in the organic matrix) nor upon the tendency to develop "spontaneous" fractures, nor upon the frequent association of dentinogenesis imperfecta and/or of otosclerosis with the disease.

SUMMARY

Both tissue-level bone formation and an index of cellular level bone formation rates were measured in 11 patients with osteogenesis imperfecta. The former averaged 309 per cent, the latter 91 per cent, of normal. These data indicate that at these levels the OGIM skeleton produces new bone in plentiful amounts. By the process of elimination and from the purely quantitative point of view, overactive resorption, not deficient bone formation, should somehow cause the subnormal accumulation of skeletal mass which characterizes this affection.

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