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# THE STUDY OF A RIB BIOPSY FROM A PATIENT WITH OSTEOGENESIS IMPERFECTA

## *A Method Using* in vivo *Tetracycline Labeling*[1]

*By*

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Undecalcified sections of a rib biopsy were studied in a woman with osteogenesis imperfecta who had been given a double tetracycline bone marker *in vivo*. Brightfield and flourescence microscopical measurements showed that bone formation inside the cortex was three times normal speed, and was fast enough to have made the rib almost three times thicker than it was had it occurred at the same rate at only one periosteal surface. Both bone formation and resorption were present on the periosteal and endosteal surfaces, yet the transverse dimensions of the rib were less than half of normal values. A specific defect confined to the bone surface drift mechanism is shown by these findings to be present in this woman.

We present here quantitative histological studies of lamellar bone tissue and cell *dynamics* in osteogenesis imperfecta, done with the aid of *in vivo* tetracycline bone labeling. This technique allows *rates* to be measured in histological sections because two markers are fixed in the bone separated by a known interval of time. Like growth rings in trees, these markers are temporally and physically stable. The amount of new tissue lying between the markers, compared to the total amount of tissue, provides a direct measure of the rate of bone formation during the labeling interval. A woman with osteogenesis imperfecta offered herself voluntarily for the study, was given a tetracycline bone marker, and a rib biopsy was done. We had hoped to find answers to some

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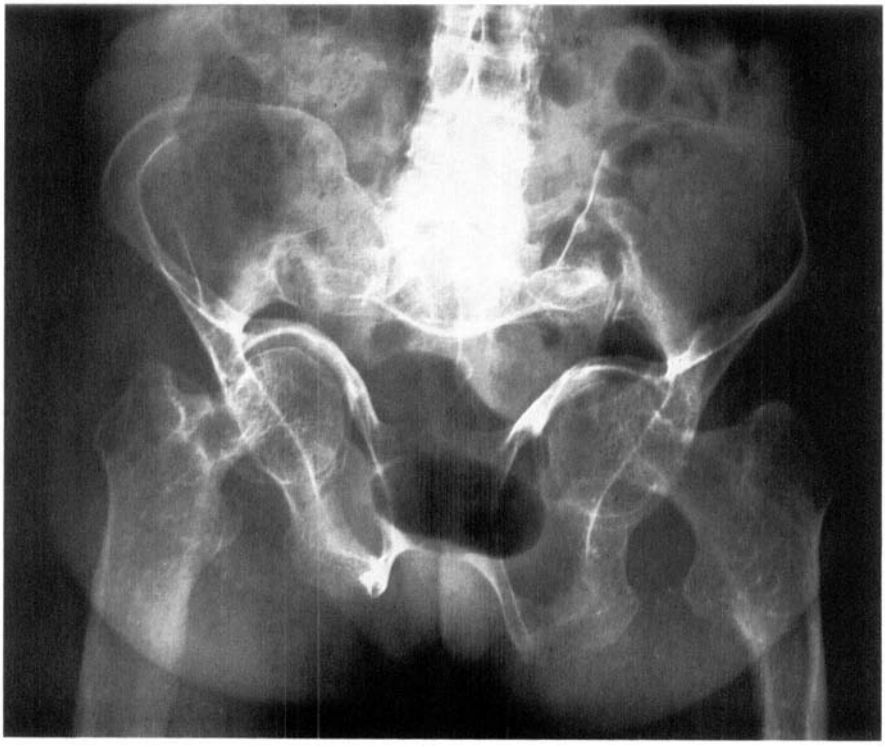

*Figure 1. An anteroposterior view of the patient's pelvis, showing the narrow femoral neck, arthrokatadysis and deformity of the obturator rings and proximal femurs.*

basic and long puzzling questions about the physiology of bone in this disease, such as, can the osteoblasts form lamellar bone, can the mesenchymal (*i.e.*, progenitor) cells make new osteoblasts, or can the osteoblasts make bone fast enough?

## MATERIALS AND METHODS

### *Materials*

The patient, a 51 year old woman, was diagnosed as having osteogenesis imperfecta at birth. She had 46 spontaneous fractures of various bones until 16 years of age, at which time spontaneous fractures stopped. In the three years preceding this study, she has been troubled with increasing back pain, and x-rays reveal minor collapses of several vertebrae which look much like those seen in other forms of symptomatic osteoporosis. The x-rays of her pelvis and femurs are reproduced in Figures 1 and 2. She has a mild degree of hearing loss due to otosclerosis.

The patient's brother (age 55, 26 fractures, otosclerosis) and sister (age 61, 17 fractures) also have the diseases, are self sufficient economically, and ceased

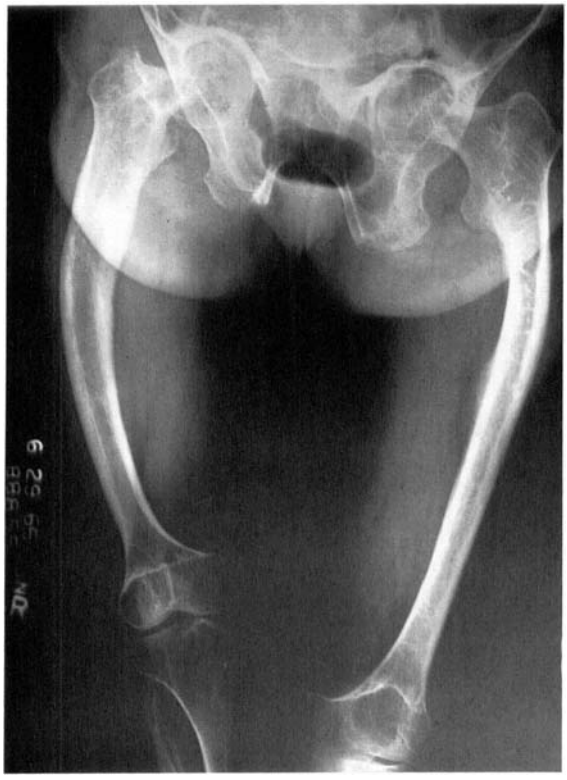

*Figure 2. An antero-posterior view of the patient's femurs, showing the bowing due to old, healed fractures, and shortening of the right femur due in part to anterior bowing which is concealed by the projection.*

having spontaneous fractures between age 12 and 17. The sister is suffering from back pain also. The parents were normal as far as the children know.

The patient's serum calcium, inorganic phosphate and alkaline phosphatase have repeatedly been found to be normal. A hemoglobin, white and differential counts, urinalysis, fasting blood sugar and blood urea nitrogen were normal. She had blue sclerae as a child but after maturity this sign gradually disappeared, as it did also in her brother and sister. The patient is 46 inches tall due to deformities caused by malunions of fractures.

*Methods.*

A) *Labeling:* The patient was given an *in vivo* label of 900 mg/day orally of demethylchlortetracycline in divided doses for *three days,* and then *ten days* later was given the drug for *six* more days (we call this the 3-10-6 label). At eleventh rib biopsy two weeks later the anterior 5 cm was taken for study (1). The quality of the rib felt normal as it was being stripped and cut during the operation.*

* In our past experience this purely qualitative observation has been a reliable clue to the presence of osteomalacic bone (soft and unusually flexible), to very low turnover bone such as is found in persons who have been treated for long

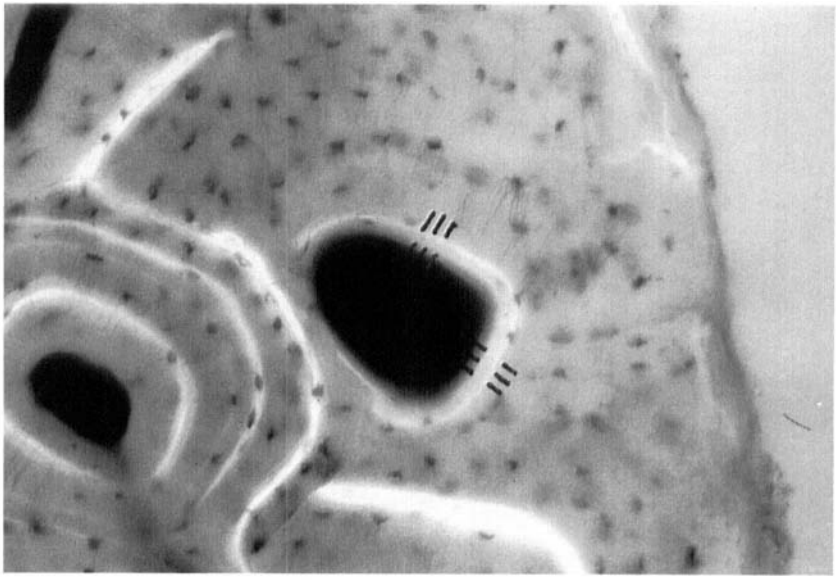

*Figure 3. Undecalcified cross section of the patient's rib biopsy at about 400 ×, taken by combined fluorescence and brightfield microscopy. The India ink bars identify the double label which we gave the patient, deposited in an osteon which was being made during the labeling period. The other bright bands are a minimum of five previous labels which she received incidentally to the treatment of infections with a tetracycline antibiotic.*

B) *Sections:* Five fresh, undecalcified, unfixed, unembedded, complete cross sections 50 microns thick were made by hand grinding, stained with the Villanueva tetrachrome bone stain, and mounted in H.S.R. (2, 3).

C) *Measurements:* We measured the cortical cross section area (precision .4 $mm^2$) (4, 5); the mean distance separating the two rings of tetracycline with a Zeiss photofluorescence microscope (precision .4 microns) (see Figure 3) (4, 6, 7); the number (relative precision 3 per cent) and average thickness (precision .8 microns) of osteoid seams, which identify sites of new bone formation within the cortex as well as on its periosteal and endosteal surfaces (8, 9); the number of resorption spaces (relative precision 3 per cent) (10); the mean thickness of the walls of the Haversian systems (precision 3 microns) (6, 11); and the mean circumference of the osteoid seams (*symbol:* $S_f$) found in actively forming Haversian systems (precision .02 mm) (12). The vertical and transverse outer diameters of the sections were measured under the stereomicroscope. These data are listed in Table 1.

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periods of time with adrenalcortical steroids (hard and brittle), and to high turnover bone such as occurs in thyrotoxicosis and acromegaly ("green", like a branch of live wood).

*Table 1. The raw data from the patient's rib, compared to age and sex comparable normals*

| | Mean OGIM | Age comarable normal |
|---|---|---|
| Cortical Cross Section Area | 5.70 $mm^2$ | 14.0 $mm^2$ |
| Distance between Tetracycline Rings (19 day label) | 12 $\mu$ | 17 $\mu$ |
| Number of Osteoid Seams per Section | 7.3 | 4.1 |
| Osteoid Seam Thickness, Mean | 7.7 $\mu$ | 8.8 |
| Per cent Perosteal Surface Labeled (Combination of 6 labels) | 22.7 | – |
| Per cent Endosteal Surface Labeled (Our label only) | 60.7 | – |
| Number of Resorption Spaces | 5 | 5.0 |
| Circumference of Osteoid Seams | .36 mm | .32 |

*Calculations:* The number of bone forming and resorbing sites are reduced to the mean per unit cortical cross section area (*symbols:* $A_f$ and $A_r$, resp.) by dividing their total numbers by the cortical cross section area (8, 13). The daily linear appositional rate (*symbol:* $M_f$) is the mean thickness of the tetracycline banding interval divided by the corresponding number of days, which was 19 (the measurements were from the outer edge of the first band to the inner edge of the second) (6, 7, 13). The bone formation rate (*symbol:* $V_f$) was obtained by: (*i*) multiplying the mean seam circumference by the mean number of seams per $mm^2$, giving the total bone forming surface in the average (*i.e.*, representative) cubic millimeter of cortical bone (13); (*ii*) then multiplying this by the mean appositional rate in millimeters per year to give the volume of new bone made in the representative cubic millimeter of rib cortex.* This may be expressed with an equation thus:

$$V_f = A_f M_f S_f \quad (1)$$

where $V_f$ is the amount of new bone made per year in the representative cubic millimeter of bone, and the other symbols have the meaning already assigned to them. The solution to this equation is a volume fractional rate constant (13).

While conversion from area to volume may seem unjustified, it is valid because of the optical sectioning principle underlying the analysis, and because of the homogeneous longitudinal grain of rib, which is studied in thin cross section (4, 14). The formation rate was then converted to the *per cent* of the cortical bone that was replaced by new bone per year, by moving the decimal two places to the right. Because of the steady state property this figure is also, within a few per cent, the

* This is analogous to finding how much new ice is made on skating rinks per city block in Detroit. Find the mean area per rink ($S_f$), then multiply by the mean number of rinks per block ($A_f$), and multiply by the average thickness of new ice made daily per rink by flooding and freezing ($M_f$).

resorption rate and the remodeling (*i.e.* turnover) *rate* (13, 15). These calculations are listed in Table 2, with age comparable normal values.

*Table 2. The calculated data for the patient are compared to sex and age comparable normals.*

| | $A_f$ | $A_r$ | $M_f$ (Microns/ day) | $S_f$ | $S_r$ | $V_f$ |
|---|---|---|---|---|---|---|
| OGIM | 1.29 | .88 | .51 (.91) | .36 | .30 | .097 (9.7%) |
| Normal | .29 | .36 | .91 | .32 | .2 | .031 |
| S.D. | ±.25 | ±.14 | ±.3 | ±.14 | ±.15 | ±.015 |
| S.E. | .03 | .02 | .08 | $< 10^{-4}$ | $< 10^{-4}$ | .003 (3.1%) |

Legend: S.D.: One standard deviation. S.E.: Standard error of the arthmetic mean. $A_f$: Seams/mm$^2$ cortical cross section area. $A_r$: Resorption cavities/mm$^2$. $M_f$: Linear appositional rate. $S_f$: Mean circumference seams. $S_f$: Mean circumference resorption cavities. $V_f$: Bone formation rate per year. OGIM: Osteogenesis imperfecta.

## RESULTS

1) In grinding the sections the bone was normally tough and was not abnormally brittle. Microscopically, there was the usual amount and a normal pattern of fuchsin permeable bone (which usually indicates low mineral desity) (16).

2) There were 1.3 osteoid seams per mm$^2$ of cortical cross section area. An age comparable normal is .29/mm$^2$ (S. D. ± .25; S. E. .03) (8, 13).

3) There were .88/mm$^2$ resorption spaces. An age comparable normal is .36/mm$^2$ (S. D. ± .14; S. E. .02) (10, 13).

4) The mean seam circumference was .36 mm. An age comparable normal is .32 mm ± .14 (S. E. $< 10^{-3}$).

5) The linear appositional rate (thickness of the layer of new bone added on older bone per time unit) was .57 microns/day or .203 mm/year on endosteal surfaces. The age comparable norm for both is .91 microns /day (S. D. ± .3; S. E. .06) or .33 mm/yr (S. D. ± .1; S. E. .02). Of the 22 osteoid seams present at the time of biopsy, only two failed to accept the tetracycline label. The appositional rate at periosteal surfaces could not be measured because the labels were too thin, and too close, and their identity (*ie.*, our label or old labels) could not be determined.

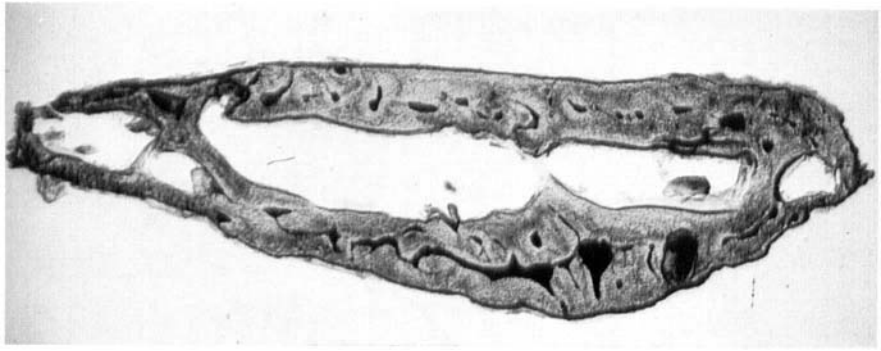

*Figure 4. The patient's rib seen in cross section, taken through the distal end where some marrow cavity was present.*

6) Twenty two per cent of the circumferential lamellae at the periosteum contained one or more of the six tetracycline labels this patient was known to have received (see Section 8 below, and Figure 5b, 5d). This reduces to 3.6 per cent per label. There was no significant difference in this measurement between the cutaneous periosteal and pleural periosteal surfaces. In contrast, 60.7 per cent of the cortical endosteal surfaces showed the double tetracycline label we gave the patient. The difference reflects the fact that endosteal surface turnover was much more rapid than periosteal surface turnover.

7) Howship's lacunae were plentiful on the periosteal bone surfaces. These are usually believed to designate sites of active or recently active bone resorption.

8) The vertical diameter of the sections was 6.9 mm, and the horizontal diameter 1.8 mm. Normal values would be 12 and 3.5 mm respectively. The cortical cross section area was 5.7 $mm^2$; a normal value is 14 $mm^2$. See Figure 4.

9) There were at least five other tetracycline labels (see Figures 3, 5) received before our contact with the patient when one of these antibiotics was given to treat infections. The large number of bands still remaining from these other labels is proof that the number of sites of osteogenesis found at the time of biopsy was representative of her rate of bone formation.

10) From a qualitative standpoint, we were unable to find any abnormality in the pattern or structure of the anisotropic and isotropic lamellae in the bone when inspected between crossed polars on the polarizing microscope.

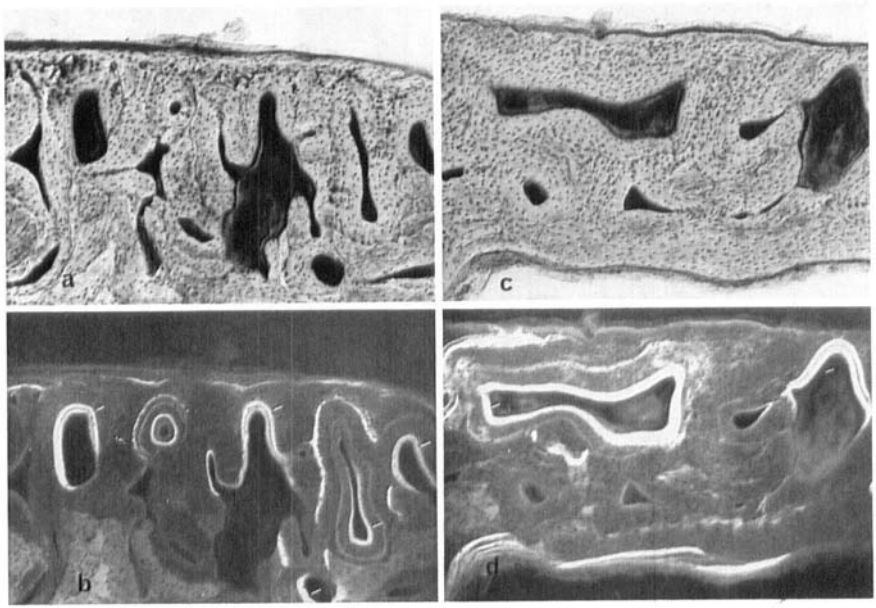


*Figure 5. a) About 200 ×, brightfield view of one of our patient's sections. b) Same field by fluorescence microscopy. The short, diagonal white markers lie each on the right side of the double label which we gave the patient. There are five of these in this field. The other bright bands are older labels of unknown time and duration. They show that the high turnover rate at the time of biopsy was representative of her usual turnover speed. The periosteal surface is at the top. It contains several old labels which are partly buried underneath a few microns of newer bone deposited on top of them. c) A different field from the same section, brightfield, about 300 ×. d) The same by fluorescence microscopy. Two of the double labels we gave lie in the middle of the section. The cortical endosteal surface lies at the bottom, and shows a series of labels, none of them at this location having been the labels we gave.*

## DISCUSSION

1) A brief review is in order of some proposals that have been made about the nature of the cellular disorder in bone in osteogenesis imperfecta.

It has been said that: (*i*) osteoblasts cannot make adequate amounts of collagen (17), (*ii*) or of organic bone matrix (18, 19, 20); (*iii*) that progenitor cells cannot make enough new osteoblasts (19); (*iv*) that the collagen is defective in structure or chemistry; (*v*) that there is an inability to replace fibrous bone with lamellar bone (2); (*vi*) that the primary defect is one of impaired periosteal and endosteal bone *formation* (20, 22); (*vii*) that the defect is an impairment of periosteal and

endosteal surface *drifts* during growth (23). As Rubin recognized (22), much of the histodynamic information about the bone in this disease was obtained from infants so badly affected that they did not survive the neonatal period. Their tissues may not provide reliable clues to the state and physiology of the bone of older children and in adults. This is largely lamellar bone and is the subject of this study.

2) In the light of these ideas, the fact that the internal remodeling (*i.e.*, cortical bone formation *plus* resorption) of this woman's rib is three times normal is very meaningful. It means (*i*) she can make lamellar bone in more than normal amounts; (*ii*) so her osteoblasts can make enough new bone matrix; (*iii*) therefore her progenitor cells can make enough new osteoblasts, and (*iv*) there is no disproportion inherent in her cells between osteoblastic and osteoblastic activity. In other words, her basic disorder* cannot be a general inability to make and resorb bone at normal speeds at the cellular level.

Caniggia, Stuart and Guideri in a superb analysis (20) proposed that new bone is not added fast enough in osteogenesis imperfecta. The tetracycline labeling technique (unavailable to those authors) shows that in this woman *this inability is limited to the periosteal surface.* The patient did add bone at nearly normal rates (0.5 microns/day) in making Haversian systems, and at quite normal rates (.9 microns/day) in laying down cortical endosteal lamellae (6, 13). If the linear rate at which she added Haversian system bone is converted to millimeters and added over the 51 years of her postnatal life, bone made at this rate at only one periosteal surface would have made a rib more than 9 mm wide medio-laterally (normal is only 3.5 mm). In other words, the capability was there, and the periosteal labels prove it, so the question is: why was this capability not used?

Transverse enlargement of long bones (including rib) during growth occurs by a special activity known as *drift* (24). It is caused by resorption (osteoclastic drift) or formation (osteoblastic drift) alone over a whole surface region of a bone. Therefore the small transverse diameters of this woman's rib are incontrovertible evidence that her periosteal drifts were impaired.

* We must study more patients to find out if this woman is representative or if this "disease" is, like osteoporosis, a sign which can be caused by a group of basically different disorders.

## CONCLUSIONS

In a woman with osteogenesis imperfecta, our studies showed: (*i*) The bone formation rate within the cortex was 3.0 times normal; (*ii*) the periosteal envelope of the rib was abnormally small; (*iii*) at the periosteal and endosteal surfaces there were a) tetracycline labeled bone forming activity, and b) Howship's lacunae indicating bone resorption which c) prove that periosteal and endosteal turnover existed; (*iv*) the appositional rate found in her osteon formation could have made her rib three times thicker than it actually was, had this rate existed at only *one* periosteal surface during her 51 years of life.

These findings reveal a single specific defect in this patient's bone: defective periosteal surface drifts. It is not clear whether this defect is inherent in the bone cells or whether these cells are responding to defective "signals" from their environment.

We believe this approach to the study of this disease offers considerable hope of clarifying the basic cellular mechanisms responsible for it. It will be necessary to study more cases, and to study bones from children with the disease, before we are ready to construct detailed hypotheses of what these cellular mechanisms might be.

## RESUME

Chez une femme avec ostéogénèse imparfaite, nos études ont montré: (1) le taux de formation osseuse à l'intérieur du cortex était de 3,0 fois plus élevé que de normale; (ii) l'enveloppe périostéale de la côte était anormalement étroite; (iii) il y avait dans les surfaces périostéales et endostéales a) une activité de formation osseuse décelée par la tétracycline, b) un manque de réaction Howship indiquant une résorption osseuse, ce qui c) prouve un renversement périostéal et endostéal; (iv) le taux d'accroissement par apposition trouvé dans la formation osseuse aurait pu rendre sa côte trois fois plus épaisse qu'elle n'était actuellement si ce taux avait existé sur *une* surface périostéale seulement durant les 51 années de sa vie.

Ces trouvailles révélent une seule défectuosité spécifique dans les os de cette malade: défaut de fonctionnement de la surface périostéale. On ne sait pas si ce défaut est rattaché aux cellules osseuses ou si ces cellules répondent à des «signaux» défectueux de leur entourage.

Nous croyons que cette approche de l'étude de cette maladie accroît considérablement l'espoir d'une clarification des mécanismes cellulaires basiques qui en sont responsables. Il sera nécessaire d'étudier d'autres

cas et d'examiner les os d'enfants qui souffrent de la maladie avant de pouvoir bâtir des hypothèses détaillées sur le rôle de ces mécanismes cellulaires.

ZUSAMMENFASSUNG

In einem weiblichen Patient mit Osteogenesis imperfecta zeigten unsere Untersuchungen:

(i) Die Geschwindigkeit der Knochenbildung innerhalb der Rinde war das dreifache des Normalen; (ii) die periostale Hülle der Rippe war abnormal klein; (iii) die periostalen und endostalen Oberflächen hier waren a) Tetracyclin markierter Knochen in Aktivitet, und b) Howships Lakunen, die Knochenresorption anzeigten, was c) beweist, dass ein periostaler und endostaler Umsatz vorhanden war; (iv) die Ansatzgeschwindigkeit ihrer Knochenbildung konnte ihre Rippe dreimal so dick gemacht haben als dies wirklich der Fall war, wenn diese Geschwindigkeit nur an *einer* Oberfläche während der 51 Jahre ihres Lebens vorhanden gewesen wäre.

Diese Befunde enthüllen einen einzelnen spezifischen Mangel im Knochen dieses Patienten: Mangelvollen periostalen Oberfächenansatz. Es ist nicht klar ob dieser Mangel in den Knochenzellen selbst liegt oder ob diese Zellen auf mangelhafte "Signale" von seiten ihrer Umgebung reagieren.

Wir glauben, dass dieser Weg zum Studium dieser Erkrankung eine bedeutende Möglichkeit darbietet, um den zu Grunde liegenden Zellmechanismus aufzuklären, der für sie verantwortlich ist. Es ist notwendig eine grössere Anzahl von Fällen zu untersuchen und auch kindliche Knochen mit dieser Krankheit zu studieren, ehe wir so weit kommen werden, dass wir eingehende Hypothesen über diese zellulären Mechanismen aufbauen können.

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