Henry Ford Hospital Medical Journal

Volume 8 | Number 2 Article 22

6-1960

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Harold M. Frost

A. R. Villanueva

H. Roth

MEASUREMENT OF BONE FORMATION IN A 57 YEAR OLD MAN BY MEANS OF TETRACYCLINES*

H. M. Frost, A. R. Villanueva and H. Roth

Until Hiroshima and Nagasaki assumed international importance, the rates and modes of bone turnover in man were primarily academic matters. The advent of atomic weapons, weapons testing, the risks attendant to their manufacture and increasing clinical use of radioisotopes made the physiology of bone turnover in man a practical problem. Research intended to illuminate mechanisms, rates and quantities associated with turnover of bone and bone-seeking substances has been hampered by limitations imposed in using man as a laboratory animal. The immediate and latent risks attending administration of radioisotopes limit the applicability of existing radioanalytical techniques to bone at the light microscopic level. The large and detailed body of knowledge gained from animal work needs qualitative and quantitative corroboration in man before it can be applied to him. As a result of this state of affairs and others undiscussed, we do not have actual measurements of bone-forming activity in man at present. Such measurements are increasingly necessary for work in the fields of radiobiology, osteoporosis of all types, osteomalacia, growth and fracture healing.

This paper amplifies basic work of Milch, Rall and Tobie.[26,27] They noted the fixation of tetracycline antibiotics in mineralizing parts of the skeleton (tetracycline bone labelling) and subsequently detected the drugs by fluorescence in undecalcified bone sections. Our contributions to their work include amplification of the optical behavior of achromycin,[12] an economical method of observing tetracycline fluorescence in bone[13] and illustration with a single human case of the kind of information which tetracycline labelling may yield.

We feel the chief value of this paper is that it presents simple, reliable, effective and precise techniques for measuring bone formation in man in vivo. With these techniques, it should be possible to determine with considerable precision the factors affecting human osteoblastic activity.

Tetracycline bone labelling has the advantages of little immediate and no known latent risk. Numerous opportunities in large medical centers occur to obtain human tetracycline labelled bone from both sexes, all ages and diverse disease states. Use of tetracyclines in human experimentation should be safe.

By virtue of their permanent fixation in mineralizing, new skeletal tissue the tetracyclines label bone forming activity current during the period of drug dosage.[12,17,26,27] Suitable preparation and examination of bones from patients who received tetracyclines at known times and for known duration permits us to measure their bone turnover more than 6 years after tetracycline administration. It is an eerie feeling to realize that one can measure precisely the osteoblastic activity going on in a bone 6 or more years before the patient died![17]

Using tetracycline labelled bone from 43 patients, and with the aid of methods largely developed in the Henry Ford Hospital Orthopaedic Research Laboratory, we

*Supported by Grant No. 293, Henry Ford Hospital.

have been able to measure in man, *in vivo:* the biological half life of Haversian bone; the biological half life of entire bones; rates, quantities and variations in remodelling activity in different bones; osteoblastic activity; and the time required to form Haversian systems.

In this paper the *methods* used for such measurements are outlined through the device of analyzing one human case. Some of the kinds of information that can be wrung from tetracycline labelling are presented for this case. Presentation of detailed measurements of large numbers of human cases, and the effects of disease on bone turnover, will be presented in separate publications.

MATERIALS

The materials studied herein are the middle thirds of the femur, tibia, fibula and entire second metatarsal of the amputated left lower extremity. Corroboratory material for some of the minor points presented in the following paragraphs are sections made as recommended from more than 40 other patients who received tetracyclines before death or surgery.[17] Clinical details of the case are presented in Appendix (A).

METHODS

Four or more complete cross sections of the listed bones were made, stained and mounted by Frost's techniques.[7,8]

Fluorescence: As noted by Milch and coworkers[26,27] refrigeration does not affect tetracycline fluorescence in bone. Material stored in bulk 8 months is unaffected. Thin sections kept in ethanol, however, gradually lose their fluorescence over many weeks. Permanent mounts made as recommended have not faded perceptibly in 3 years. Tetracycline fixed in bone in vivo does fade perceptibly after 6 years of life.[12]

The microfluorescence equipment used by Milch and coworkers is excellent. Fluorescence can also be energized by a 430 mu band in the visual spectrum. A set-up for this method is Wratten 47 and 47B filters in series in the light source and Wratten 8 and 9 filters in series over the eyepiece. The light source should be a high intensity tungsten source such as a 300 watt 35 mm slide projector. Kohler illumination is superior to others with this arrangement.[1] Tetracyclines fixed in tissue fluoresce strongly with this arrangement. Their color is white to gold depending on drug concentration and section thickness. Background is a faint green or magenta. Fluorescence may be proved by passing the Wratten 8 filter under the condenser, whereupon fluorescence abruptly ceases. The condenser should be racked close to the slide and the aperture stop be wide open. For further details consult reference (13). See Figure 2.

Yellow color in bright field Microscopy: Achromycin[a] in achromycin-labelled sections prepared as recommended[7,8] develops a lemon yellow color within one to 14 days of mounting (in Harleco synthetic resin-xylene).[12] Failure to stain and mount as recommended usually prevents this color from appearing. The color does not develop in bulk material. Once developed it appears to be permanent as long as

(a) All of the currently available tetracycline antibiotics, singly or in combination, are nearly equally suitable for work of the type described in this paper.

the mount is preserved. The visible yellow areas correspond precisely to the fluorescent areas. Very thin planes of achromycin labelling, or zones labelled during time of low dosage, may not develop detectable yellow in bright field but will fluoresce.

Appearance of tetracycline labelled bone: Labelled bone appears as yellow-stained bands, the bands being parallel to and in the substance of lamellae. Bands do not cross lamellae or cement lines. The drug deposits in mineralizing bone and cartilage and appears fixed there by subsequent mineralization. No diffusion out of labelled volumes of bone has been seen in mounts 3 years old or in bulk material 8 months old. The labelled band-width is a direct function of *duration* of dosage and *rate* of mineralization of osteoid while intensity of fluorescence and visual yellow is a function of dosage per day. When life continues after cessation of dosage the labelled bone and calcified cartilage become buried by unlabelled bone and calcified cartilage unless appositional growth ceased during drug administration. During the period of tetracycline administration most tissues are stained by the drug. Within 48 hours of drug cessation "adventitious" tetracycline has disappeared from bone, leaving only the labelled bone and calcified cartilage behind as a tell-tale. This and other observations suggest to us that tetracycline staining of bone is ordinarily a readily reversible phenomenon due at least in part to the ability of tetracycline antibiotics to complex with calcium ions. For further details consult references (12, 26, 27).

Permanent staining of Haversian canals, osteocyte lacunae in Haversian systems and calcified cartilage (which were mature during the period of drug administration) has not been seen.[12,17,20]

Observation of the visual yellow in tetracycline labelled undecalcified sections is aided by use of light corrected to 5000°K, apochromats and fully illuminated objectives.[3,28]

Photomicrography: The visual yellow in brightfield is due to fluorescence of tetracycline energized by the blue end of the visible spectrum and so cannot be photographed on ordinary black and white emulsions; excellent color photomicrographs with Ektachrome, and black and white with fluorescence, can be obtained.[4,6] (See Figs. 1 and 2).

MEASUREMENT METHODOLOGY

In order to measure bone turnover in man, we must be able to measure the amount of new bone formed in a unit volume of bone in a unit of time. Tetracyclines administered *in vivo* conveniently label bone actively forming, and conveniently they do not label unmineralized osteoid or already mineralized bone. The tetracycline labelling process supplies the same type of information one would obtain by feeding large amounts of alizarin to man, but without the toxicity associated with alizarin.

In the following material in the *Methodology* section, the actual measurements required are outlined. In the *Data* Section, the manner in which the measurements are manipulated to provide useful information is outlined. We make extensive reference to other publications to shorten an already lengthy paper.

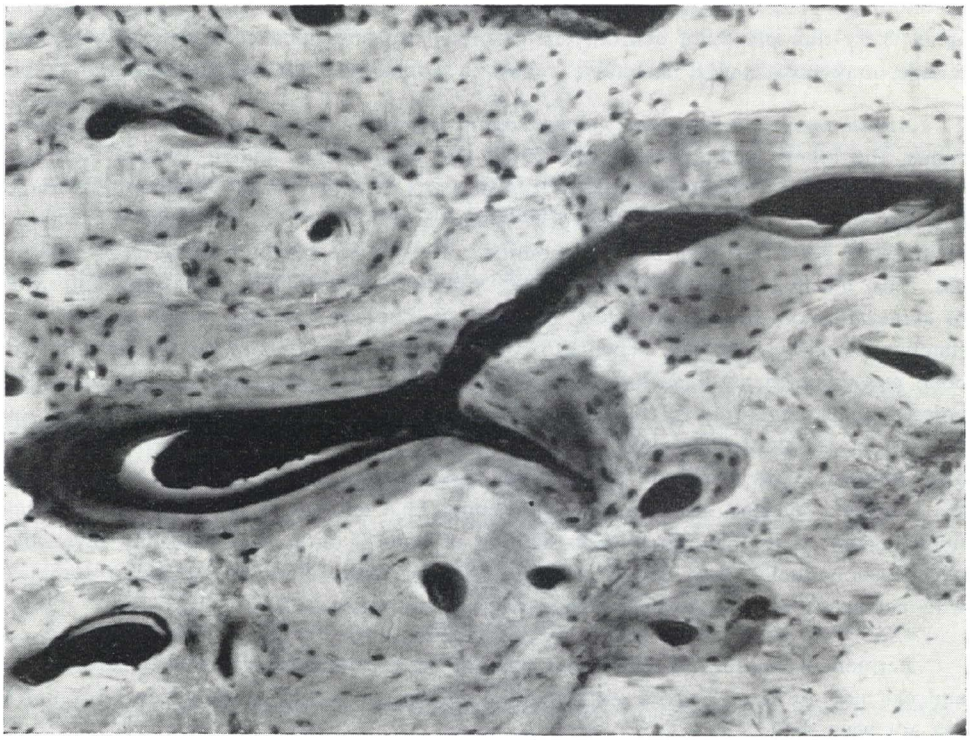

Figure 1

Photomicrograph, Cross Section, femur, basic fuchsin, undecalcified, of the case presented in the text. Brightfield, 200X. The black dots are vascular channels. The dark zone connecting the Haversian channel at left midcenter to the Haversian channel at 2:00 o'clock is a Volkmann's canal which is out of focus. The two Haversian channels are lying in newly formed Haversian systems and are still fuchsin permeable in some areas, causing them to appear darker than the remainder of the bone visible in this illustration. Parts of 3 Haversian systems banded by achromycin and pronouncedly yellow on the original section are in this field but are not demonstrable on the photograph for reasons referred to in the text.

Because of known distributional differences in various parts of single bones, complete cross sections were necessary for the following measurements.[10,11,15,18,19] The longitudinal grain of bone is associated with much less distributional variation along the long axis than is found along the radial and tangential axes. Longitudinal sections also provide too limited a sample. We emphasize here, and will reemphasize later, that the present measurements are done on the diaphyses of 4 bones of one patient and must not be considered representative of norms for the sex and age of all similar people.

The means of measurement used for the data in Charts I and II are now presented.

1. *Channels/mm²:* The slide was marked with a grid of lines which are scanned in increments of one field diameter, using an objective-eyepiece combination of known field diameter. The total numbers of longitudinally coursing vascular channels and of fields are recorded and reduced to channels/mm² by calculation. Relative variation in the present case was small, about 4%. Sampling

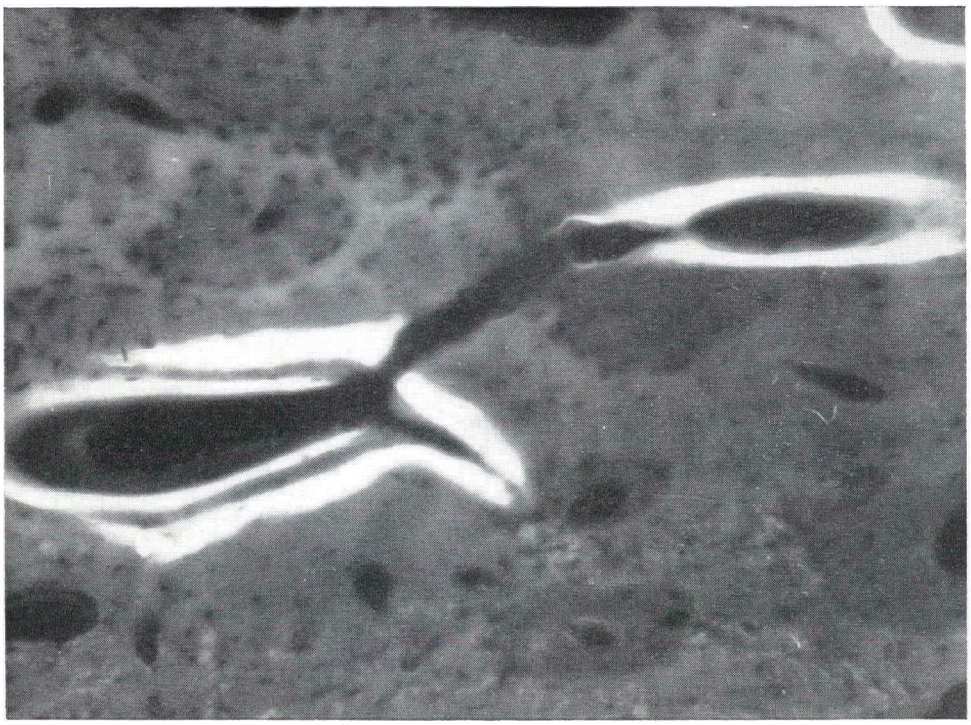

Figure 2

Same field and magnification but with Frost's fluorescence set-up. The bright bands are achromycin labelled parts of the Haversian systems. The left hand system is doubly banded, the outer band being formed during the first, and the inner band during the second achromycin epoch of the case discussed in the text. The right hand Haversian system is singly banded and it is not apparent from the original material whether the band formed during the first or second epoch.

technique must be properly designed or systematic distributional effects inherent in bone and the observer will introduce error. For example there are 2 to 3 times as many channels near periosteal surfaces as near endosteal, while osteon density and incidence of banded osteons is greater near bone angles than in broad bone surfaces. For full details consult references (18, 19).

2. *Percent osteons*[a] *labelled; labelled osteons per section; percent osteoid seams; percent seams in labelled osteons:* Counts are done as in (1) using a fully illuminated low power apochromat and color corrected light, tabulating counts during the process with the aid of a hand counter. The complete sections, rather than a sample, were scanned for labelled osteons per section, using the mechanical stage to avoid under—or over lap. (Consult references 12, 18, 19, for details of the measurement and for explanation of osteoid seams and seam counts).

3. *Osteons banded in outer, middle, inner thirds or doubly banded; banded osteon lying in outer, middle or inner thirds of cortices:* At any one time

(a) An osteon is an Haversian system.

MEASURED DATA

Chart I

Measured Characteristic	Femur	Tibia	Fibula	Metatarsal
Longitudinal vascular channels/mm²	13.2	14.1	12.2	16.2
% Osteons Banded (Number counted)	2.1 5207	0.65 2000	2.4 1448	1.06 662
Number banded osteons per section, average of 3 or more	149	26	24.5	9
% banded osteons with osteoid seam	4.7	3.8	24.5	11.1
Double banded osteons as % all banded osteons	11.1	11.5		11.1
Outer Third of Osteon Banded	26.1	21.7	35.1	28.6
Middle Third of Osteon Banded	25.8	30.4	25.6	28.6
Inner Third of Osteon Banded	47.0	47.8	38.4	43.3
Unclassifiable	11.0	11.7	20.6	22.2
Banded Osteons Outer Third Cortex	56.3	95.7	84.3	100
Banded Osteons Middle Third Cortex	26.3	4.2	15.6	0
Banded Osteons Inner Third Cortex	17.3	0	0	0

MEASURED DATA

Chart II

Measured Characteristic	Femur
% Osteons Containing Osteoid Seam at Amputation (2100 counted)	0.098
Average Osteon Width	80 u
Range	18.5 - 135 u
Average Width Labelled Bands,	19.9 u
Unselected Range	3.1 - 30 u
Average Width Outer and Middle Third Bands	26.0 u
Average Width Inner Third Bands	6.0 u
% Section Surface Vascular Channel	10%
% Section Surface Banded	.58
% Empty Lacunae, Banded Osteons	2.1
% Empty Lacunae, Inner Third Band of Doubly Banded Osteons	0.5%
% Empty Lacunae, Outer Third Band of Doubly Banded Osteons	2.3%
% Empty Lacunae, Mature Haversian Systems	18.5
% Empty Lacunae, Extra-Haversian	55.7

CALCULATED DATA: SEE TEXT

Chart III

DATA	Femur	Tibia	Fibula	Metatarsal
Osteons/Banded Osteon	45.4	154	41.4	94.3
Banded Osteons/mm²	0.290	0.091	0.294	0.176
Mean Osteon Formation Time	5 wks.	5 wks.	5 wks.	5 wks.
Osteoid Seam Thickness Formed/Day	2.4 u	—	—	—
Instantaneous % Osteoid Seams	0.75	0.23	0.86	0.38
Instantaneous Quiescent Osteons/Mineralizing Osteon	125	430	115	262
Biological Half Life, Entire Cortex				
Years	7.6	24.2	7.6	12.6
Days	2700	8600	2700	4500
Biological Half Life, Osteons				
Years	2.7	8.6	3.1	5.6
Days	960	3000	1100	2000
% Osteons Remodelled per Year	18.7	5.9	21.4	9.4
% Mass (and osseous volume less vascular and cell volume) Mineralizing/Day	0.018	0.0058	0.018	0.011
% Mass in Turnover/Day	0.036	0.012	0.036	0.022
Ratio of Remodelling Activity per Unit Volume (Femur=100)	100	31	101	61
Ratio of Remodelling Activity in Terms Total Mass in Turnover (Femur=100)	100	19	15	7.5
% Remodelling Activity in Outer Third Cortex	56.3	95.7	84.3	100

in a given patient, some osteons will be in the initial stages of formation, some in the final stages and some in between. Labelling with tetracycline will accordingly appear, in the subsequently completed osten, as a band in the outer, middle or inner third of the osteon. If two periods of drug dosage were close enough together, then some osteons would exhibit double bands, one within the other as in Figure 2. Banded osteons were tabulated separately by completely scanning sections of femur, tibia, fibula and mentatarsal with a low power apochromat. 344 banded osteons were counted for these measurements. Doubly banded osteons were counted twice. Of the total banded osteons, 11% could not be classified as to the third of the osteon banded *so these figures do not add up to 100%.*

4. *Average width[a] of osteon; average widths of labelled bands:* Banded osteons were selected at random and measured with a calibrated eye-piece micrometer.[3,28] The totals were reduced to arithmetic means. 49 osteons were measured for average osteon width; 29 unselected bands, 29 outer or middle third bands and 29 inner third bands for average band widths. The average osteon *diameter* is more than twice the listed value since the vascular canal was ignored. The average would be about 240 u with a range of 40-300 u.[17]

5. *Percent empty lacunae:* Reference[11] discusses the technique utilized in these measurements. Briefly lacunae without stained contents are empty and dead. More empty lacunae exist in extra-Haversian than in Haversian bone[b]. The

(a) Width here refers to the distance between cement line and Haversian canal along a radial axis. See Figs. 1 and 2.

inference is that increasing age of patient and bone moiety increases the counts of empty lacunae. Lacunae opening into the section surfaces are not counted. High numerical aperture is essential for the counts.

6. *Percent section surface area:* The theory of this technique is that in a well mixed, multiphase solid, an infinitely thin plane cut through it will contain proportionate surface areas of each phase in direct proportion to the volume components of each phase in the solid, provided the area of the plane is sufficiently large to avoid random distributional effects. This ideal plane may in turn be sampled by running the cross-hairs of a filar micrometer across it, or by other means. Enough runs to reduce random distributional effects below the desired level must be made. Consult references 15, 16, 19, 22, 23, for fuller details.

   The Leitz integrating eyepiece micrometer was designed for the above type of measurement. The microscope objective is used to provide the measured plane, and means for mechanically adding the various phase constituents measured are built into the instrument. The femur was selected for the measurements because of its greater homogeneity as compared to the other bones. For example, the relative variation in counts of total labelled osteons per section was only 0.6% in the femur, 4% in the tibia, 10% in the fibula and 20% in the metatarsal. This suggests that when enough cross section area has been sampled the results are valid for adjacent parts of a diaphysis, and that more sections of small bones must be measured to achieve meaningful results.[19]

   In the present integrating eyepiece measurements, 12 fields were measured at 150 X and 0.32 N.A. with full illumination of the objective. The estimated probable error (a combination of instrument, resolving power, optical, section thickness and operator effects)[3,4,19,21,22,28] is plus or minus 10% for the vascular and 40% for the banded areas.

7. The volume of lacunae and canaliculae is 2.5% of the cortex exclusive of vascular volume. This measurement is detailed in reference 16.

8. Charts I and II list the values obtained for the measurements described above. The data in Chart III are calculated from the data in Charts I and II. See following text for the reasoning involved.

DATA ANALYSIS

1. *Osteon turnover:* Several informative calculations can be made from the data in Charts I and II. These calculations tell us how many osteons are being formed per unit time and per unit volume of diaphyseal bone. They also tell us the proportion of the total osteon population undergoing remodelling per unit time. From this the biological half-life of the osteon population may be caculated for each diaphysis measured.

(b) Haversian bone as defined for the present purpose consists of intact osteons only. In double or triple systems only the innermost one is considered Haversian. The remainder of the cortex is designated extra-Haversian and includes circumferential lamellae and fragments of old osteons.

(A) During the 40 days of drug administration in this case, the percentage of the total number of osteons mineralizing was: femur 2.1%; tibia 0.65%; fibula 2.4%; metatarsal 1.06%.

(B) The number of quiescent (fully formed) osteons per mineralizing osteon were: femur 45.4; tibia 154; fibula 41.1; metatarsal 94.3.

(C) From the vascular channels/mm^2 for each bone the banded osteons/mm^2, thus osteons/mm^2 mineralizing in 40 days, may be calculated: femur 0.29; tibia 0.091; fibula 0.29; metatarsal 0.18. Reasoning elaborated elsewhere reveals that these figures are representative of mineralizing osteons/mm^3 also.

(D) Since 40 days is 0.113 years the proportion of osteons undergoing mineralization in a year can be calculated: femur 18.7%; tibia 5.9%; fibula 21.4%; metatarsal 9.4%.

(E) The biological half-life of osteons may now be estimated. Note that osteons comprise about 0.3 of the total cortices[17] and that a different half-life for the entire cortex will be calculated from direct measurement. Osteon half-lives are: femur 2.7; tibia 8.5; fibula 2.4; metatarsal 5.3, all in years.

(F) Several striking features emerge from the above measurements: the long half-life of osteons, the similarity of the fibula and femur in remodelling activity in this patient, the decrease in remodelling activity as examination proceeds from proximal to distal (suggesting that in the axial skeleton higher rates occur in this patient) and the remarkable inactivity of the tibia. One wonders if the tibial values reflect a general characteristic or if unrecognized disease affected this bone, but not the fibula, in this patient.

It is reasonably assumed that as much bone is broken down as is formed in a given unit time, barring certain pathological states. If this be true then the calculations in (1) should also apply to the rates of bone destruction.

2. *Osteon formation time:* Here we will determine how long, on the average, it took for an osteon to form in this patient in the bones examined. Two separate approaches to the problem are possible in the present case. One is based on the frequency distribution of banded osteons, the other on a simple proportional measurement of width of the bands and width of the osteons.

(A) About 11% of the total banded osteons were doubly banded, first in the outer, then in the inner third of the osteon's width. See Figure 2. The outer bands average 20 u, the inner bands 6 u thick. A mean time interval between labelling epochs of 7 weeks will be assumed. This is the time during which all of the doubly banded osteons formed. If this were the *mean* time for *all* osteons there should be about as

many that formed faster than 7 weeks as formed slower. The faster ones would exhibit one band but the slower ones would exhibit two bands, *one of which was in the middle third of the osteon.* Osteons singly banded in either middle or outer thirds constitute about 40% of the *total* but there were only 3 doubly banded osteons one of whose bands was in the middle third, in 344 counted. This suggests that the *mean osteon formation time* is less than 7 weeks. A period of about 5 weeks will be assumed because of the following reasoning.

(B) The average osteon width was 80 u. The average width of the middle and outer third achromycin bands in doubly banded osteons was 26 u. These bands were labelled during a drug administration time of 11 days. 80/26 X 11 should be the time of osteon formation. This is 34 days. It is not certain that ignoring the first 3 days of drug administration is justified or that the rate of osteon formation is steady, however, matters which we have assumed to be the case for the moment. Nevertheless the evidence is compatible with an osteon formation time of about 5 weeks in this patient.

(C) Assuming 5 weeks to be correct, 2.4 u of osteoid a day were elaborated in mineralizing osteons in this patient.

3. *Instantaneous percentage of osteoid seams;*[9,18,19] This is the percent longitudinal vascular channels containing mineralizing bone and thus a seam at any one moment. It is assumed here that the percentage of osteoid seams and the percentage of labelled osteons are equivalent. Subsequent publications will document this assumption.

(A) Assume osteon formation rate and numbers were equal during the first and initial part of the second drug administration periods. Then half the singly banded osteons were labelled in the first 11 day period. During 11 days some osteons completed, while others commenced, formation, causing some overlap. The overlap should be about 11/35 of the instantaneous value. On this basis the value of osteons forming at any one moment for the femur is 0.75%. The value found at amputation was 0.1%. This indicates that the vascular events preceding gangrene markedly retarded new osteoid formation. The reader should note that the somewhat unrealistic assumptions made here are *unfavorable* to the qualitative result derived. The retardation in bone forming rate which occurred during the second achromycin administration epoch was probably *greater* by about 40% than the figure we quote.

(B) Using a conversion factor of 0.36[a], the incidence of banded osteons can be converted to supply the instantaneous value of osteoid seams. Chart III lists them.

4. *Turnover of entire diaphysis:* Because there are marked variations in the proportions of Haversian to extra-Haversian bone in various bones and in

(a) 11 days drug dosage÷35 days osteon formation time.

various ages, determination of osteon turnover or half-life does not tell us about turnover or half-life of the entire bone cross-section. Tetracycline labelling permits a direct measurement of entire cross-section turnover to be made by planimetric means.

(A) A representative femoral cross-section was measured with the Leitz integrating eyepiece micrometer. 10% of the area was vascular channel and 0.58% was banded by achromycin. The volume of osteocytes and canaliculae is roughly 2%.

Thus while 0.58% of the total cortical volume was banded, 0.63% of the dry, fat-free cortical mass was banded, assuming uniform bone density, by 40 days of achromycin dosage.[b]

We calculate that 0.018% of this femur was mineralized through new bone formation per day. According to previous reasoning this is also the amount undergoing destruction per day. If the patient's vascular difficulties retarded bone formation during the second achromycin dosage period, this figure and those below are too small by a factor of, at most, 40%.

(B) If the percent of mass undergoing mineralization per day is dependent on the percentage of banded osteons, estimates of mineralizing mass per day can be calculated for the other bones by simple proportion. Values are: tibia 0.0058%; fibula 0.018%; metatarsal 0.011%.

(C) Bone turnover as utilized here means the sum total of bone being formed and degraded. This is twice the formation quantity. The values per day: femur 0.036%; tibia 0.012%; fibula 0.036%; metatarsal 0.022%. These are precent of mass, or percent of osseous volume excluding vascular and cellular volume. These measurements assume a normal balance between bone formation and destruction, something probably not present in the particular case we present. Another, direct means of measuring bone resorption quantity and rate is needed to evaluate bone turnover in disease states such as osteomalacia and senile osteoporosis.

(D) The remodelling rates and quantities of the various bones may now be compared with each other. In terms of mass per unit volume turned over, with the femur set arbitrarily at 100, the values are: femur 100; tibia 19; fibula 101; metatarsal 61.

In terms of the absolute mass turning over in the middle thirds of the bones, using the banded osteons per complete cross-section as a means of proportional comparison, and with the femur set arbitrarily at 100, the values are: femur 100; tibia 19; fibula 15; metatarsal 7.5. In these calculations we assume there is no significant difference in the

(b) Bone density is not uniform at the macroscopic or microscopic levels but it is permissible at present to assume a uniform average density. For some work on density variations consult references 29, 31.

size of osteons in the various bones. If high precision is required, this assumption would require documentation by measurements on the separate bones.

(E) The mean half-life of the entire cortex may now be calculated, using percent of mass mineralizing per day as the base. The values are, in years: femur 7.6; tibia 24.2; fibula 7.6; metatarsal 12.6.

(F) It will be noted that the osteon half-life is about 0.3 the above values. This suggests that there are different turnover rates for Haversian and extra-Haversian bone.

This inference is substantiated by finding appreciable portions of circumferential lamellae in extra-Haversian bone even in very old patients. Since circumferential lamellae form during appositional growth in all phases of childhood, to be later partially replaced by osteons, it is probable that the circumferential lamellae in this patient's extra-Haversian bone is older than the half-life values suggest, in some instances probably 40 years old. It may be noted here that micropetrosis affects extra-Haversian bone, particularly circumferential lamellae, long before it occurs in Haversian bone. Micropetrosis is related to the biological age of the bone affected and is not found in young bone.[11] These points also suggest a much longer half-life for extra-Haversian than for Haversian bone.

5. *Locus of remodelling activity:* The distribution of banded osteons in the cortical thirds is inhomogeneous. Most of the remodelling activity occurs in the outer third of the cortex. In the femur, correction for the difference in vascular channels/mm^2 between periosteal and endosteal surfaces balances the difference out, but this correction does not balance out the preference for outer third remodelling activity in the rest of the bones. These facts pose two questions: why should remodelling activity concentrate near the periosteal surfaces, and why are there more vascular channels there than elsewhere? While a peculiarity of periosteal blood supply might be postulated, it is also possible that the greater surface stresses and strains play a role. This is a problem which should prove amenable to experiment using tetracycline labelling as an investigative method.

6. The case we present offers an opportunity to check the thesis that one of the factors causing bone cells to die in vivo[10] is age. We performed counts of percent empty lacunae in this case which reveal:

Banded osteons (known to be younger than 95 days) had an average of 2.1% empty lacunae.

The inner band of doubly banded osteons had 0.5%; the outer band 2.3% empty lacunae. (Larger differences in empty lacunae between central and peripheral lacunae usually occur in human Haversian systems so this is a poor index of how many cells might die in 95 days).

Mature, normally mineralized osteons exhibit an average of 18.5% empty lacunae.

Extra-Haversian bone exhibits an average of 55.7% empty lacunae, but with variations. Micropetrotic bone for example comprises about 10% of the femur (on which these counts were done) but is 100% dead. It is noteworthy that no banded micropetrotic areas were seen in any of the 20 sections scanned for this purpose or in any of the 42 other cases available to us which are labelled in vivo with tetracyclines.

It appears that the empty-lacuna counting technique might serve as a means of assigning relative ages to various parts of the same bone, extraneous factors being absent. In this case a definite correlation between age of the bone and percent empty lacunae exists.

DISCUSSION

A.) We wish to emphasize that the foregoing measurements and calculations are valid only for the diaphyses of the bones of the case examined. Extrapolations to the whole bone or skeleton or to man as a whole are not justified. There is reason to expect that in the future factors of equivalence may be determined which will permit such extrapolation.[5] Such factors would prove invaluable in skeletal research and clinical work.

B.) The behavior of tetracyclines is not necessarily representative of the behavior of all bone-seeking substances. Differences in ionic size, sterism, charge, solubility, dielectric constant, chemistry, hydration shells and other factors make such similarity unlikely. Nevertheless it is possible in some instances that equivalence factors are determinable which would permit the behavior of tetracyclines and some other hypothetical substance to be equated. Some likely candidates, with the qualifications below in mind, are lead, strontium, radium and calcium.

As an example of how misleading the figures on the amount of bone labelled per day by achromycin may be, consider the following: Partly published work[8,9,18] reveals that in mineralizing osteons containing an osteoid seam there is a thick zone of permeability beyond the seam to diverse ions and large molecules. The zone thickness usually exceeds 15 u and represents the instantaneous volume of the mineralizing osteon which is readily permeable to these substances. Achromycin, however, deposits only in a thin band at the periphery of the seam,[12] affecting a volume of bone smaller by a factor of about 20. Such a difference in behavior reqiures explanation and makes one cautious about assuming permeability similarities in two different substances.

C.) This paper deals with only one aspect of bone turnover as that term is commonly understood, and that is bone formation and bone destruction. These are not the only mechanisms by which bone-seeking substances may enter or leave the skeleton, and therefore not the only important mechanisms involved in radiobiological turnover and exchange work with bone. It is now possible, however, to assign turnover, in the sense used by us, some

quantitative meaning so that other modes of turnover may be placed in perspective.

D.) The techniques which we have utilized in this work, and the manner in which we have applied these techniques, should pave the way for accurate measurement of normal osteoblastic activity in man. This is because the newly mineralizing bone labelled by tetracyclines must be formed by osteoblasts, and measuring the product is equivalent to measuring the production rate directly.

E.) With the above means it should be possible to determine what and how various factors affect osteoblastic activity. For example, do estrogens really accelerate osteoblastic activity in man, or do they exert a different effect? How do the corticoids, thyroxin and growth hormone act and what is their relative importance? These are some of the questions we hope to be able to answer in the future. It is astonishing how little hard, factual knowledge exists at present and serves as a basis for a large edifice of clinical theory concerning osteoblastic activity.[1,2]

F.) When the factors affecting osteoblastic activity in man have been recognized through work on the lines suggested above, the clinician will be able to *cure* osteoporosis of any type, to affect bone growth in any desired manner and to cause fractures to heal more dependably and in less time than is currently required. Much work, of course, lies between us and such capabilities.

G.) The bone formation studied in the present case was *lamellar* bone formation. It has been pointed out elsewhere that fibrous and lamellar bone osteoblastic activity differ in some manner in their chemical processes and in their regulatory mechanisms.[14] In past experimental work on osteoblastic activity, failure to distinguish between these two fundamental bone types necessarily invalidates the interpretation of such research.[1,2]

SUMMARY

In vivo tetracycline labelling of bones from one patient, from which undecalcified sections of the femur, tibia, fibula and metatarsal were made, permitted the *direct* measurement or estimation in a 57 year old man of the following aspects of bone physiology:

Osteon turnover rate and half-life in the various bones examined.

Total diaphyseal bone turnover rate and half-life in the various bones examined.

Mean time of osteon formation.

Locus of the major part of bone remodelling activity.

Comparison of turnover rates in different bones.

Quantitation of bone formation and destruction per unit time and volume in the bones examined.

The techniques used in making the above determinations offer new means of studying human bone physiology. Within their limitations of scope, they should prove

useful in advancing our knowledge of skeletal dynamics.

The data presented in this paper are the first time such direct measurements have been made in man.

APPENDIX (A)

CASE PRESENTATION

Briefly, a 57 year old Caucasian male developed an aortic aneurysm which was resected and replaced with a prosthesis. An interval of good health followed. Then infection and leakage, after several temporizing surgical procedures, led to complete ligation of the blood supply to the left lower extremity, gangrene and pertrochanteric amputation. These events occurred during two separate hospitalizations in each of which the patient received known amounts of achromycin for known time periods.

There were no objective or subjective circulatory difficulties in his lower extremities until a thrombophlebitis of 5 days duration appeared on the 48th day after first achromycin administration. Transient circulatory difficulty appeared on the 75th day after a surgical procedure. Gangrene occurred on the 97th day after ligation of all collaterals to the limb and extirpation of the entire left limb of the arterial prosthesis. Amputation was done on the 102nd day. The amputated limb provided the material for the present study.

Achromycin was administered in dosage of 2.0 gm/day on the following days, numbering them from the day of first administration 1-3; 11-21; 63-88. No achromycin was given on the other days. These periods will be referred to subsequently as the first and second achromycin epochs, days 1-3 being ignored.

Multiple other antibiotics were administered but no other tetracyclines. (All of the other antibiotics and the tetracyclines have been tested in vitro for bone staining; only the tetracyclines fluoresce and stain undecalcified bone visibly.)

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