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Lamellar Osteoid Mineralized Per Day In Man

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LAMELLAR OSTEOID MINERALIZED PER DAY IN MAN*

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INTRODUCTION

The basic observation by Milch, Rall and Tobie that tetracyclines become fixed in and label newly mineralizing bone, and may subsequently be detected in undecalcified sections by microfluorescence,[14,15] has provided us with a potent new investigative tool to be used in the study of human bone physiology in vivo. Some aspects of the methodology involved with their use have been discussed elsewhere.[6,8,12,13] In this paper measurements of the thickness of new Haversian osteoid mineralized per day in a small group of patients will be presented. The measurements were made on undecalcified bone sections with the aid of tetracycline antibiotics given the patients at varying times before death or surgery.

When a patient receives tetracyclines, most of his body tissues become stained with the drug and remain so as long as the drugs are continued. Upon stopping the tetracyclines, the tissues destain. One exception to this destaining is the skeleton. Tetracyclines produce a peculiar stain at the outer edge of osteoid seams where progressive mineralization is occurring, and this stain is permanent in that it remains until the bone is resorbed by remodelling processes. Accordingly, bone which has been "labelled" with tetracycline in vivo at any time in the past contains the information of time, location, quantity and rate in its tetracycline label. This need only be read by suitable means after surgical resection or postmortem sampling to yield information on the osteoblastic activity of the patient during the time he was receiving the tetracycline antibiotic. In the Henry Ford Hospital Orthopaedic Research Laboratory, specimens are available which reveal the presence of tetracyclines given at known times and duration as much as 7 years prior to sampling of the skeleton.

If the duration of tetracycline administration is known, then it should be possible to measure the thickness of new bone being mineralized per day by dividing the width of the labelled band by the number of days over which the tetracyclines were given. It should also be possible to tell from such measurements whether or not there is any significant variation in rate of mineralization and whether or not the zone of tetracycline instantaneous staining is a sharp plane or a broad band.

In this paper measurements of the thickness of new, Haversian, lamellar bone mineralized per day are given. Circumferential lamellar bone formation is given in only one case (958200), and fibrous osteoblastic activity is deliberately excluded from this study.[7]

MATERIALS

Nineteen bones from 14 patients were examined. These patients all received tetracycline antibiotics at known times and for known duration prior to death or operation. The patients are listed in Table I along with other pertinent data. Patients known to have received androgenic, estrogenic or corticosteroid hormones have been excluded from this study, with the exception of case 854185 who received corticoids

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the last 3 weeks of life. Patients with moderate or severe congestive heart failure have also been excluded from this study. The reason for these exclusions is that osteoblastic activity as determined by several means is markedly affected by the hormones or disease state referred to and their effects on osteoblastic activity will be published separately.

CASE	Age	Sex	Duration Tetracycline dosage in days	Total average width tetracycline bands (u)	Range, width tetracycline bands (u)	Uncorrected osteoid mineralized per day (u)	Corrected osteoid mineralized per day	Spec. obtained OR or PM	Bones examined and # sections measured	Tetracycline given # months before PM or OR
198963	64	M	17	11.9	5-20	0.7	0.46	PM	Clav. 6	1.5
062070	56	F	15	18.7	9-25	1.25	0.98	PM	Clav.-Rib 6-6	1
243010	68	F	6	9.3	3-20	1.55	0.89	PM	Clav. 3	48
343203	70	M	5	7.3	3-9-12	1.4	0.66	PM	Rib 2	19
389217	61	F	10	14.7	5-30	1.5	1.07	PM	Clav.-Rib 3-2	72
529754	12	M	3	5.1	2-13	1.7	0.37	PM	Clav. 4	0.1
822604	35	F	5.5	5.9	2-9	0.98	0.35	O.R.	Rib 4	7
854185*	66	M	0.7 2	4.5 4.2	4-5 2-10	6.4 2.1	0.7 0.1	PM	Rib 4	0.03 20
855929	15	F	8	8.9	4-15	1.1	0.61	PM	Clav. 2	11
890793	62	M	10	5.9	2-10	.59	0.2	PM	Clav. 3	0.3
954439	15	M	5	9.5	4-16	1.9	0.9	PM	Rib 4	0.6
958882	52	F	7	7.8	3-14	0.9	0.54	PM	Clav.-Rib 2-2	0.03
943014	57	M	11 18	24 39	5-55 15-60	2.2 2.2	1.8 1.9	O.R.	Fem-Tib-Fib 9 Rib	3.0 1.7
958200	0.5	M	6	15.5	7-35	2.6	2.6	PM	3	0.9

Table I

METHODS

Fresh, undecalcified, complete cross sections of the bones examined were made, stained and mounted by the writer's methods.[1,2] The writer's simple microfluorescence set-up was utilized for visualization and measurement of the labelled bone.[6]

Measurements of the widths of tetracycline labelled bands in Haversian system (circumferential lamellae in case 958200) were made with a calibrated eyepiece micrometer at sufficient magnification and N. A. so that the only major error was estimation of the limits of the tetracycline band in the sections. An uncertainty of plus-minus 2

micra affects the latter estimation. Fifteen or more measurements on successive, labelled Haversian systems were made and averaged in each case. This average is tabulated in Table I as the average width of the tetracycline band. Measurements were not made in areas where the labelled structure was oriented obliquely to the optical axis of the microscope.

Time and duration of tetracycline dosage was obtained from hospital and OPD records.

The amount of osteoid mineralizing per day is obtained by division of the band width by the number of days of dosage.

OBSERVATIONS

From the data in Table I the following is either directly indicated or can reasonably be deduced.

From the range in width of the labelled bands in individual cases it appears that the rate of mineralization of new osteoid is a very variable thing and that this variation is a normal characteristic. We must assume that the osteoblastic activity which produces the osteoid being mineralized is subject to similar variation in rate. Seemingly some local factors exist which affect the rate of osteoblastic activity locally.

The average thickness of mineralized new osteoid per day for the whole group is about .9 microns. Considering the size of the group and the number of cases, the group differences obtained by averaging the results according to age, sex, and proximity in time to surgery or fatal illness are not significant. More cases and more measurements are needed before such comparisons would be statistically justified.

Case 854185 was included (in spite of corticoid therapy) because tetracycline was administered for less than a day. Yet the width of the tetracycline band in the labelled Haversian systems was 4.5 microns. This, plus comparison of the band widths in the other cases given tetracyclines for 3 days or less, indicates that at any one moment in time a band thickness of about 3-4 microns is being stained. This is the zone of tetracycline deposition. This zone moves centripetally a certain amount during each day of tetracycline administration. The instantaneous locus of the permanent tetracycline staining in mineralizing Haversian bone and circumferential lamellae is thus a 3-4 micron thick band, not an infinitely thin plane.

For the present, the writer adopts the value of 4 microns as the thickness of this instantaneous staining band. When more data are available this value may need to be increased or decreased by one micron or less. The value probably varies in individuals.

On the above basis Table I contains a column in which the corrected amount of osteoid mineralized per day is calculated. The calculation is simple: 4 microns are subtracted from the total thickness of the tetracycline band; the remainder is divided by the number of days of drug dosage.

Regardless of the value chosen for the momentary width of the tetracycline staining band, correction of the measurements for the value reveals that there are

considerable differences in values from patient to patient. These differences are too large to be errors in methodology. They reflect real variations among the patients.

DISCUSSION

It will be possible to calculate the volume turnover of bone, at least in diaphyses, by applying the above data to the geometry and to average measurements of diameter and length of Haversian systems in man, accounting in the calculations for the variation in numbers of Haversian systems with age and per unit area cross-section of bone and per unit volume of bone. Such determinations, being essentially numerical, will provide a good check on the planimetric methods used previously by the writer.[8]

Once we have good measures of normal bone remodelling activity, we may then apply these methods towards the investigation of osteoblastic activity and mineralization rates in various diseases, in particular senile and postmenopausal osteoporosis.

The measurement methodology outlined in this paper has potential as a means of investigating local and systemic factors controlling osteoblastic activity in both man and animal. The method is capable of considerable precision, something heretofore lacking in investigations of osteoblastic activity in man and animal. The reasons for previous lack of precision in the experimental and observational investigations of osteoblastic activity done to date are numerous. Included are the failure of previous workers with radioisotopes to recognize and correct for feathering,[5] average minerali-

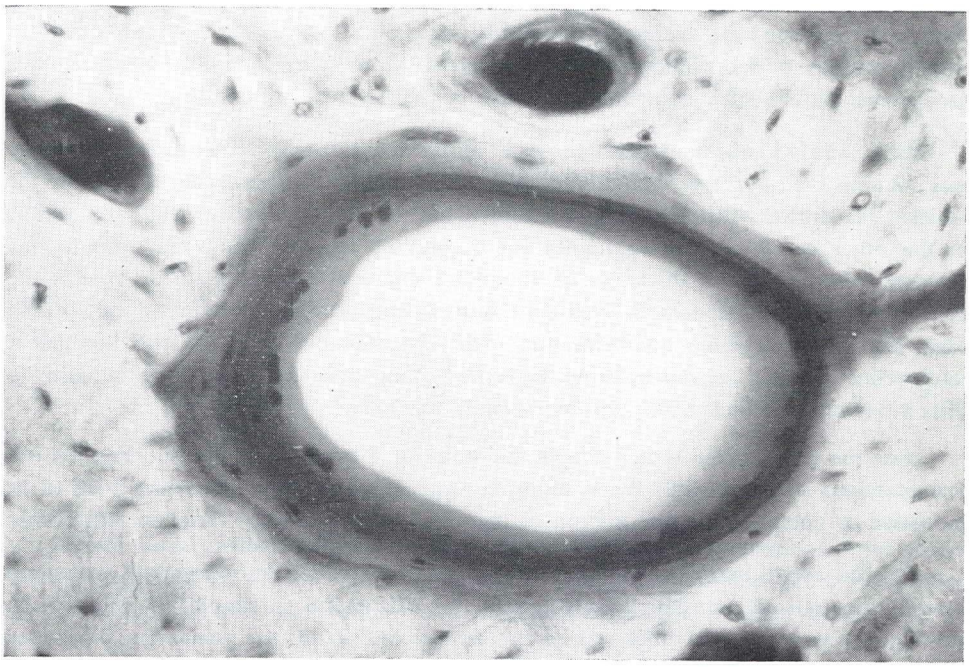

Figure 1

Clavicle; undecalcified, fuchsin stained section of patient who had received a tetracycline antibiotic up to 12 days before death. Administration of the drug then stopped so that in the 12 days preceding death, no tetracyclines were given. An osteoid seam lines the Haversian canal. Bright field.

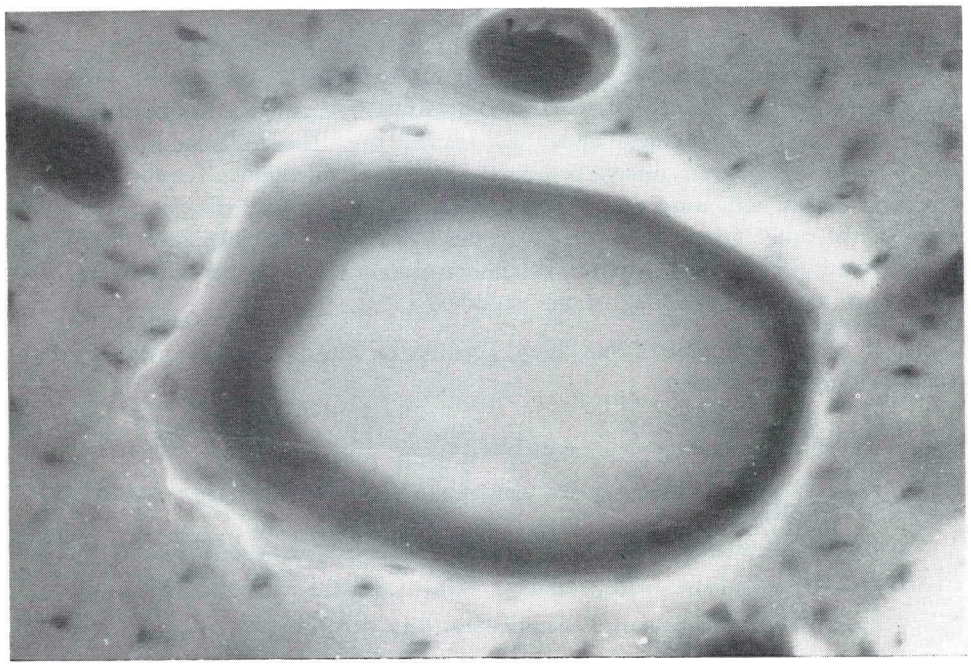

Figure 2

Same field but with writer's fluorescent set-up. An osteoid seam lines the central Haversian canal. At 9:00 o'clock it has grown 15 u centripetally from the bright band (the bright band is tetracycline fluorescing in already mineralized bone). The amount of osteoid mineralized per day in this part of the osteon is about 0.9 u when calculated as suggested in the test. At 12 and 1 o'clock the seam is in a resting state. There has been no significant mineralization of osteoid in this part of the seam in the 12 days before death of the patient. The seam lies directly on tetracycline labelled bone without any intervening unlabelled bone.

zation density, average cell death,,[3] normal distributional differential effects in man and animal[4,5,8,9] and failure to account for halo volume[9] and micropetrosis.[4] Measurements utilizing non-radioactive methodology have suffered from lack of technical development[8,11] and lack of knowledge of osteoblastic qualitative physiology.[5,7,8]

The variations in rate of mineralization of new osteoid illustrated in Table I are almost certainly the result of similar variations in rate of osteoblastic activity in the same zones. Such variations indicate the existence of local factors which affect osteoblastic activity in addition to the known (and postulated) systemic factors which affect—i. e.: regulate—osteoblastic activity. The methods used in this work would permit investigation of these local factors to be done, with the prospect that we might eventually learn how to make fractures in adults heal in weeks rather than in the months they do require. Such an objective will not be easily achieved, however! The reader should understand that initial fracture healing is accomplished by fibrous bone, and should realize that fibrous bone osteoblastic activity is subject to different local and systemic controls than lamellar bone osteoblastic activity.[7] Failure to make this distinction previously has seriously confused thought and work on the osteoblastic aspects of fracture healing, bone graft acceptance and the various forms of osteoporosis.

SUMMARY

Using in vivo tetracycline "labelling" of mineralizing, newly forming bone in a group of 14 humans, the thickness of osteoid mineralized per day has been measured on cross-sections and averages about 0.9 micron/day. The instantaneously staining part of newly mineralizing osteoid is a band about 4 microns thick. Tetracycline which is present outside this band during dosage with the tetracyclines disappears with cessation of the drug. Tetracycline present in this band is permanently fixed in place up to the 6 year period which was the maximum observed interval between dosage with the drug in vivo and sampling of the skeleton.

This is the first time it has been possible to measure directly an aspect of osteoblastic activity in man.

REFERENCES

1. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:272, 1958.

2. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

3. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

4. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

5. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

6. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

7. Frost, H. M.: Observation on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1950.

8. Frost, H. M.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

9. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. (In press).

10. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

11. Frost, H. M., Villanueva, A. R., and Roth, H.: Halo volume, Henry Ford Hosp. M. Bull. 8:228, 1960.

12. Frost, H. M., Villanueva, A. R., and Roth, H.: In vivo staining of bone with tetracyclines, Stain Technol. 35:135, 1960.

13. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracycline antibiotics, Henry Ford Hosp. M. Bull. 8:255, 1960.

14. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

15. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.