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THE CROSS SECTION SIZE OF THE OSTEON

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Introduction

It has been suggested that there is an abnormality in the physiology of lamellar bone in patients with diabetes mellitus.[1] We have reported finding a major decrease in the rate at which the radius decreases in the average, actively forming osteon or Haversian system in patients with this disease.[2] This has been called the radial rate of osteon closure. Yet, when the decimal part of the actively forming osteon that has been completed is plotted against the corresponding decimal part of its total formation time that has elapsed, the curves thus obtained seem to be the same in both normal and diabetic people.[3,4]

It could not be concluded from the study of the radial rate of osteon closure referred to that the rate of formation of osteonal bone in toto is decreased in diabetes. In part, this is because the size of the diabetic osteon was unknown. It is theoretically possible to increase the rate of production of osteonal bone in the whole body by increasing the average size of the osteon, as long as other things are kept equal.

In this study, the mean cross sectional size of the osteon is determined in samples of bone from both normal and diabetic subjects. This is necessary for accurate interpretation of previous (and subsequent) studies of diabetic bone. There are additional reasons for studying osteon physiology. The production of new osteons may be regarded as a special case of the more general problems of the synthesis of extracellular protein, particularly of collagen since bone matrix is about 95 per cent collagen.[6] Osteon formation occurs in an easily measureable volume of tissue space, it involves the production of a well defined amount of new protein, it requires a measureable period of time, and it involves a measureable number of cells.[5]

Materials

The material was obtained from two populations: normal and diabetic people.

1. The middle third of the 5th, 6th or 7th rib was obtained from 10 patients in each age decade from birth to age 80,* making a total of 80 ribs from 80 people. All of these people were metabolically normal. Most of them came to autopsy for causes such as trauma, suicide, acute poisoning and homicide. One quarter of them supplied rib at

*We wish to express our gratitude to Drs. E. S. Zawadski and R. Horn for this material, and for the opportunity to abstract the clinical and autopsy records involved.

thoracotomy for indications such as patent ductus arteriosus, biopsy of solid parenchymal lesions and correction of hiatus hernia. None of them had a known chronic illness or diabetes mellitus. Autopsy and/or clinical records were made available to us in all cases.

2. Bone from the same sampling site was obtained from a second group of 20 patients with known diabetes mellitus, most of whom were considered to be under adequate management with insulin or tolbutamide. Their ages ranged from 20 to 70 and their mean age was 52 years. Some of them had a serious illness superimposed on their diabetes. Specifically, some of them had gangrene, congestive heart failure or chronic infection, and one of them had pyelonephritis. But the average osteon in the adult's skeleton is at least 10 years old if one accepts the data of Sedlin and Frost obtained from four people[6], and it is about 15 years old if one accepts the bone turnover figures obtained by Frost in a study of 57 tetracycline labelled people[7].*

The rationale for collecting this particular sample of bone is threefold. First, ribs are one of the few bones from metabolically normal people which are available in quantity (at autopsy or elective thoracotomy). Second, they can be removed freely at autopsy without offending undertakers or families. Third, a reliable and valid skeletal sampling procedure has to cope with these known facts: the cell activities vary in different bones from the same skeleton, although in a characteristic way; the cell activities vary in different parts of the same bone, although in a characteristic way; the cell activities vary at different times during the year in the same bone, so that averages obtained from measuring many separate cases are needed to obtain representative and valid group means. These facts mean that the sampling site should be constant, as must section orientation, in order to get valid samples of various groups of people[8,9].

Selecting the same part of the diaphysis of the same bone has proven to be one valid way of sampling the skeleton, at least for some purposes[8].

The case material is listed according to age and sex in Table I. In Table II, the diabetic cases are listed individually with their most pertinent clinical facts.

Methods

Sections

Mineralized, accurately oriented, 50 micron thick, specially stained cross sections were made from the ribs[10,11]. Two things were measured on the sections: the total area of bone inside of the cement lines of intact, completed osteons (partially remodeled and actively

Table I

Distribution of cases by Sex and Age.

AGE	NORMAL		DIABETIC	
	M	F	M	F
0	7	3		
10	6	4		
20	7	3	1	1
30	4	6	1	1
40	5	5	2	1
50	8	2	3	4
60	9	1	2	1
70	9	1	1	2
TOTAL	55	25	10	10

*And was therefore made long before the terminal illness and agonal event.

Table II

DIABETES

CASE No.	AGE	SEX	RACE	CAUSE of DEATH or OP.	PM. or Biopsy	Ill or No
301869	29	M	W	Uremia, ASHD, CHF.	PM.	Ill
63-1145	20	F	N	Incomplete Abort.	PM.	No
929523	32	F	W	Myo. Inf.-Cor. A.S.	PM.	No
63-265	35	M	N	Lobar Pneumonia	PM.	No
359715	46	M	W	RHD; Mitral-v-tomy.	PM.	Ill
1088595	42	M	W	Metastasis Hypernephroma	PM.	Ill
988441	49	F	W	Mit. Sten. RHD Commisurotomy	OR.	Ill
958882	52	F	W	CA. Epiglottis Bronchopneumonia	PM.	Ill
970954	52	F	W	RHD-Mitral Sten. Commisurotomy	PM.	No
1002499	58	F	W	CA. Lung Pneumonectomy	OR.	No
63-415	52	F	N	Acute Eth. Alcohol Intoxication	PM.	No
63-1061	53	M	W	Car Accident	PM.	No
63-441	56	M	W	Car Accident - Chr. Pyelonephritis	PM.	No
989221	55	M	N	CA. Lung Pneumonectomy	PM.	Ill
955962	60	M	W	CA. Lung Lobecotomy	OR.	No
949935	60	M	W	Uremia Renal ART-Throm.	PM.	Ill
270758	65	F	W	Histoplasmosis Pneumonectomy	OR.	No
1095286	73	F	W	Metas. CA. Lung C-R Insuff.	PM.	Ill
376985	77	M	W	Cerebral Infarct Embolism	PM.	No
653588	75	F	W	Perf. Gastric Ulcer	PM.	Ill

List of the 20 diabetic cases with the most important clinical facts at post mortem or at the time of operation. PM: post mortem. ASHD: arteriosclerotic heart disease. CHF: congestive heart failure. RHD: rheumatic heart disease. Myo. Inf.: myocardial infarct. Eth.: ethyl. OR: operation (thoracotomy).

forming osteons were not measured), and the area of the Haversian canal. See Figure 1. Every osteon in each of two complete cross sections of each rib was measured. There were 200 sections which contained a total of 12,871 intact osteons.

The area measurements were done with a Zeiss integrating eyepiece I according to procedures outlined by Hennig[12]. These procedures are based on a measuring technique described by Delesse in 1847[13].

The method is a point counting technique and has been described previously[4]. In the Zeiss eyepiece, there are a total of 25 possible hits, so that one hit represents 4 per cent of the area of the field contained in the eyepiece. The hits on osteons were recorded on a hand talley. There were a total of over 190,000 hits in the whole study. One throw per osteon was made, so that there were as many throws as osteons, i.e., 12,871. The number of osteons measured was recorded on aother talley. The average hits per osteon was then calculated by performing the indicated division procedure, and converted to the absolute mean osteonal cross section area in mm^2 for the section using this equation:

$$A = \frac{H\ Ag}{T\ 25} \qquad (1)$$

where A is the desired area, H the total hits, T the total throws and A_g the area of the reticule grid in the object space.

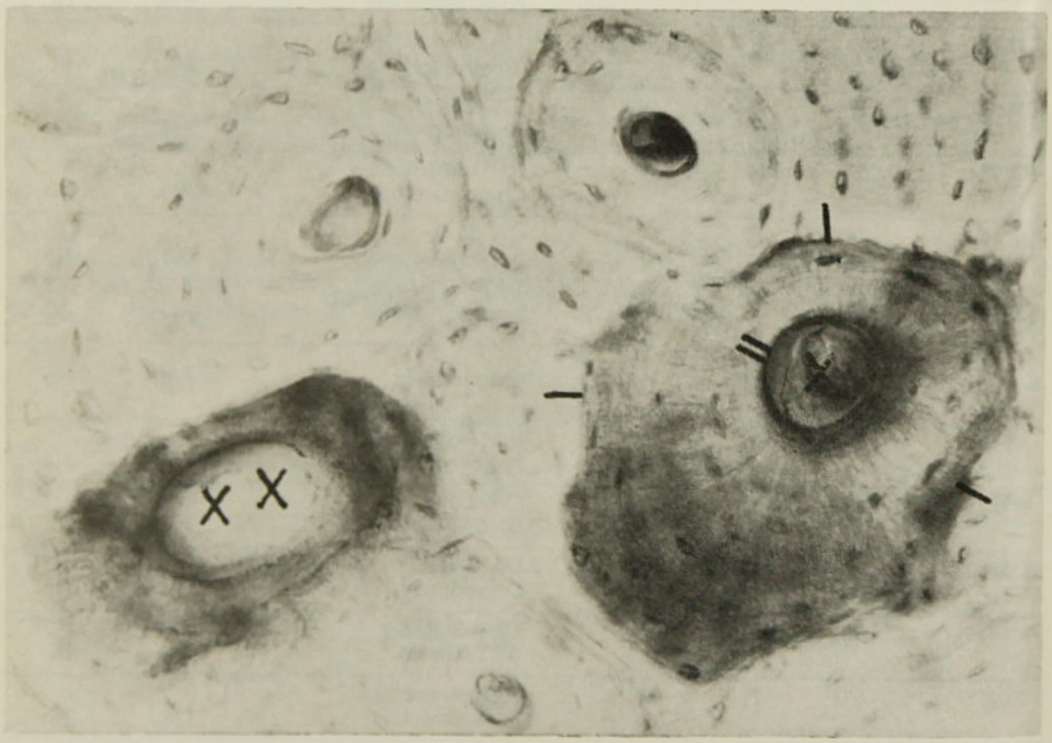

Figure 1

Mineralized, cross section of human cortical bone, 300X, basic fuchsin. The X marks identify Haversian canals seen in cross section. They are tubes with cylindrical geometry. The double X identifies an osteon that is in the process of active formation, as shown by an osteoid seam lining the wall of the Haversian canal. Such osteons were not measured in this study.

The double India ink marks identify the wall of the Haversian canal. This wall separates the lumen within from the mineralized bone matrix that lies peripheral to it.

The single India ink bars identify the cement line, which is the place where the resorptive process, which created the space for the new osteon, stopped. The irregular cross section geometry means that cross section area measurements are more dependable measures of the transverse sizes of osteons than are measurements of their diameters or radius.

Osteons are tubular, cylindrical structures about 1 mm. long[14], and they were measured in cross section. The sections on which they were measured averaged 50 microns in thickness which is much less than the length of the osteon. Therefore, the mean cross section area of a large number of osteons is a valid index of the amount of bone in some arbitrary length of the average osteon, and comparing cross section areas of osteons from one group of people to another is a valid way of comparing the sizes of their osteons. It would be desireable to know the length of the osteons also, but solving the geometrical and methodological problems that such a study poses would require a prohibitive expenditure of labor and time. It can be stated with some assurance that any systematic change in osteon length with age is small, and on the same order of magnitude as that shown in this report for the cross section area.

The mean of the measurements of the two sections in each case was next calculated and recorded. The cases were then grouped in age decades and their decade means, standard deviations and standard errors were calculated. This material is summarized in Table III. In this table are listed the values for the cross section area of bone in the osteons (space inside the cement line minus the space inside the Haversian canal), and the cross section area of the Haversian canals. This material is also shown in graphic form in Figure 3.

The design of this procedure is such that although the area of any single osteon is known with a poor degree of confidence, the mean area of the osteon and Haversian canal for a given decade is known with a high degree of confidence.*

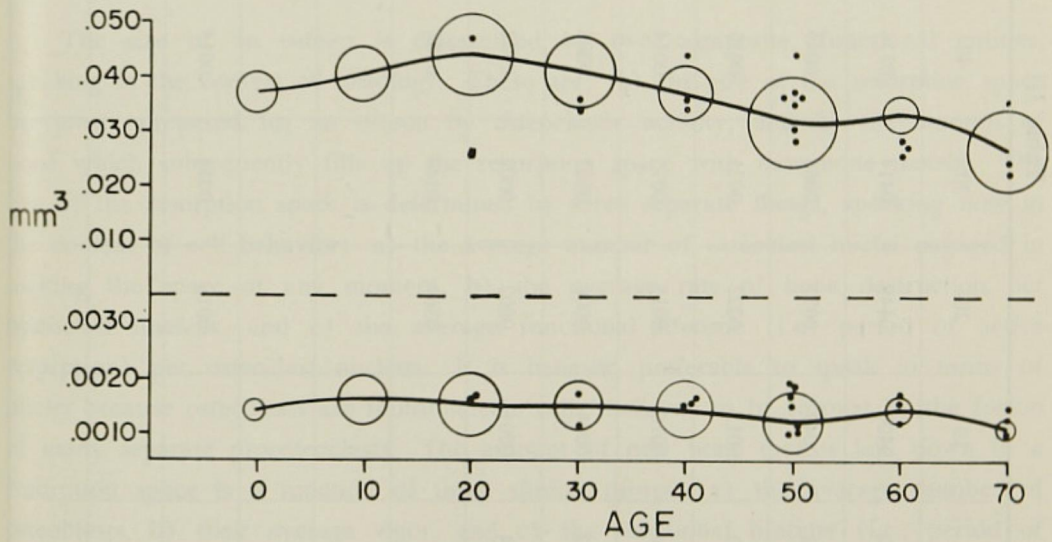

Figure 2

The measurements are shown in graphic form. The circles represent one standard deviation. The lines connect the measurements of the normal people. The individual points are a scatter plot showing how the 20 diabetics studied compared to the normal group. The mm^3 figure on the y axis indicates the volume of bone in the average lmm. length of osteon.

Table III

Average measurement by decade, with standard deviation and standard error, of Haversian systems and Haversian canals. Comparative study of 20 diabetic and 80 non-diabetic ribs. The figures represent square millimeters.

*i.e., ± 4% at 2 standard deviations.

Table III

	AGE	0	10	20	30	40	50	60	70	Mean
NORMAL RIBS	H.S. Area	.0352	.0400	.0449	.0414	.0374	.0330	.0338	.0279	.0367
	S.D.	.0041	.0047	.0071	.0068	.0049	.0076	.0033	.0066	.0076
	S.E.	.0013	.0015	.0022	.0021	.0015	.0024	.0010	.0021	.0009
	H.C. Area	.0014	.0016	.0015	.0015	.0014	.0013	.0014	.0011	.0014
	S.D.	.0002	.0004	.0006	.0005	.0006	.0005	.0003	.0002	.0004
	S.E.	.00004	.0001	.0002	.0002	.0002	.0002	.0001	.0001	.0001
DIABETIC RIBS	H.S. Area			.0369	.0348	.0388	.0359	.0273	.0283	.0339
	S.D.									.0067
	S.E.									.0015
	H.C. Area			.0016	.0014	.0015	.0015	.0015	.0013	.0015
	S.D.									.0003
	S.E.									.0001

Results

The cross section area of the osteon, less the area of its Haversian canal, is 0.036 ± 0.008 mm² for normal, and 0.034 ± 0.007 mm² for diabetic subjects. The difference between the two is not significant.

The cross section area of the Haversian canal is 0.0014 ± 0.0004 mm² in normal, and 0.0015 ± 0.0003 mm² in diabetic subjects. The difference between the two is not significant.

There is an age related variation in these values of about 10 per cent in magnitude, the peak sizes of both osteon and Haversian canal areas occurring at about age 20.

Discussion

The size of an osteon is determined by two composite, functional entities, speaking in the context of histology. These are: 1) the size of the resorption space previously prepared for an osteon by osteoclastic activity, and 2) the amount of bone which subsequently fills up the resorption space with new bone matrix. The size of the resorption space is determined by three separate things, speaking now in the context of cell behavior: a) the average number of osteoclast nuclei engaged in making the space at any moment, b) the average rate of bone destruction per osteoclast nucleus, and c) the average functional lifetime (i.e., period of active resorption) per osteoclast nucleus. It is believed preferable to speak in terms of nuclei because osteoclasts are multinucleate cells, believed to be formed by the fusion of many separate proosteoclasts. The amount of new bone that is laid down in a resorption space is a function of three similar things: a) the average number of osteoblasts, b) their average vigor, and c) the functional lifetime (i.e., period of active bone formation) of the average osteoblast.

These two composite functional entities appear to be similar with respect to their quantitative result (i.e., the amounts of bone resorbed and formed in making an osteon) in normal and in diabetic patients. This is surprising only if it is viewed alongside of the report that the rate at which the radius of the forming osteon decreases in diabetes is decreased to 36 per cent of normal.[2] This suggests that there is a separate functional mechanism in the local cell system which determines how much bone there will be in the average osteon. This mechanism seems to be to a considerable degree functionally independent of the mechanism that determines how long it will take to make this amount of bone. Were this conclusion based solely on the report

of Landeros and Frost[2] one might suspect it. However, further support appears in the form of a major, age-related prolongation in the radial rate of osteon closure, reported independently in a tetracycline-based study of 19 nondiabetic cases.[15] A decline of 40 per cent was observed between ages 7.5 and 47 years. This raises a number of very basic questions about the nature of cell controls, questions whose consideration will be deferred.

If the average size of the diabetic osteon is normal, then the meaning of the decrease of its linear rate of closure in diabetics is that it takes 2.8 times longer (i.e., $100 \div 36 = 2.8$) to make a diabetic osteon than it takes to make a normal one. The normal time required to make an osteon in ribs has been reported elsewhere to be about 80 days, or 0.22 years at age 43.[15]

Extrapolating, it would take a normal man aged 65 some 95 days or 0.26 years to complete an osteon. Therefore it takes the diabetic person of the same age 0.26 X 2.8 or 0.73 years to make an osteon.[2,16] This represents a considerable prolongation in osteon formation time in diabetes and suggests strongly that the rate of bone formation might be depressed in this disease. Additional information would be needed about the total number of osteons being formed at any moment to confirm this.

Summary

The mean cross section areas were measured of 12,000 osteons from 80 metabolically normal and 20 diabetic patients. The osteons were seen in mineralized sections cut from the middle third of the 5th, 6th or 7th rib. The mean areas for the normal and diabetic osteons were .037 $\pm$ .008 mm^2 and .034 $\pm$ .007 mm^2 respectively, excluding the Haversian canal, and .0014 $\pm$ .0004 mm^2 and .0015 $\pm$ .003 mm^2 for the Haversian canals respectively. There was a small, systematic change of $\pm$ 10 per cent across the span of 70 years of life that were encompassed in the normal group of cases.

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