Quantitative Microradiographic Study on the Mineralization and Trabecular Density of Human Vertebral Body

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The grade and distribution of mineralization in the postmortal human vertebral body were examined with the technique of quantitative microradiography. The grade of mineralization was indicated by the value of absorption of x-ray through a tested bone, and the trabecular density was shown by the number and width of the trabeculae on the microradiograms. Correlations between the grade of mineralization or trabecular density and ages or pathological conditions were statistically studied.

The grade of mineralization in respective parts of the vertebral body was apt to show almost the same pattern in each case. The trabecular density reached the maximum value in the third decade of life, and thereafter it was lowered by aging, particularly after the fifth decade.

The present study has also revealed that the vertebral body in cases of lung cancer and renal insufficiency has high grade of mineralization, whereas that in liver cirrhosis is lower than normal.

In order to know the grade of mineralization of the bone, ash content in bone tissue is usually measured. However, a technique of quantitative microradiography has recently been developed by Engström¹⁻⁴ for the determination of the grade of mineralization. This technique has greatly contributed to the studies of bone grafting (Homstrand⁵), process of fracture healing (Nilsson⁶) and changes in mineralization of the normal femur by ages (Sissons *et al.*,⁷ Jowsey⁸) and osteoporotic femur (Vose,⁹ Jowsey and Cohn¹⁰).

There are numerous reports about biochemical analysis on homogenized bone in reference to its mineral content. However, only a few works have been done with respect to the quantitative determination of the mineral in bone tissue, particularly in the vertebral body at microscopic level. The author attempted to determine the grade of mineralization and trabecular density of the vertebral body in normal and pathological conditions employing the technique of quantitative microradiography.

MATERIALS AND METHODS

The specimens were taken from postmortal vertebral bodies of the lumbar

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portion, mainly the fourth or fifth lumbar vertebra, and prepared as slices approximately 1 cm thick in a sagittal plane. After dehydration in absolute alcohol, the sliced specimens were embedded in methacrylate resin. Embedded bone was further sliced in 0.2 mm thickness with an electric saw¹¹ and ground finally to approximately 80μ in thickness, which is suitable for quantitative microradiography.

This thin bone slice was submitted to roentgenography by placing the specimen in contact with a fine-grained x-ray film and exposing it to monochromatized x-ray according to the method of Engström. An x-ray diffraction tube with copper target, a product of Toshiba Electric Company, was used in a condition of 20 kV and 10 mA with nickel filter of 20μ in thickness. The wave length of x-ray was so adjusted that more than 80% of the total emission had a wave length between 1.50 and 1.60 Å. This meets practically the requirement of monochromatic x-ray for the present investigation. The nature of x-ray is a type of $\text{CuK}\alpha$ and the wave length at the peak is 1.54 Å as illustrated in Fig. 1. The intensity of

Fig. 1. Distribution of x-ray by wave length emitted from copper target.

irradiation was regulated in such a way that the photographic density of processed x-ray film at the area of the specimen to be tested was between 0.8 and 1.4. Since the density is too high at the area directly exposed to x-ray for photometry, a small piece of copper foil of 30μ in thickness was used for roentgenograms on the same film. The photographic density of directly exposed area was calculated from the value on the copper foil and the extrapolation of the diagram was done as shown in Fig. 2. The time of exposure was constantly controlled at 70 minutes. The spectroscopic film, type 649-0, a product of Kodak Company, was used in this photography

Fig. 2. The photographic density of the film irradiated by copper target x-ray tube with and without a copper foil filter and its relation to exposure time under a condition of 20 kV and 10 mA.

and the process of development was done strictly under the same condition all the time. The photometry of the microradiogram was made with a microspectroscopic photometer, a product of Olympus Optical Company, of which pin-hole corresponds to an area of 12.6μ in diameter on microradiogram. The thickness of the specimen was measured with an electronic micrometer, a product of Anritsu Electric Company.

Linear absorption coefficient (per cm) was calculated from the obtained values as described above. This coefficient represents proportionally the grade of mineralization of bone, provided that calcium salt is in the form of hydroxyapatite in the bone.

The trabecular density was determined by measuring the spaces projected on the microradiogram from bone trabeculae in the following way: the number and width of the trabeculae on the horizontal lines, of which four penetrate each vertebral body in equal distances, were measured (Fig. 3). At first, the number of trabeculae across a horizontal line of given length was counted and the width of each trabecula was summed up. When the axis of trabecula does not meet with a given line at rectangle, the width of trabecula was measured in the rectangular direction. The average of trabecular width was then calculated. Total width of trabeculae on a given length is referred to as the density of bone trabecula in medullary bone.

These measurements were done on postmortal vertebral bodies of 137 cases in total. All the specimens were obtained from the vertebrae without macroscopically demonstrable destruction. Thirty-four cases of death due to accident were

Fig. 3 A

Legends

Fig. 3A. Microradiogram of a vertebral body in an 80μ thick vertical section. The number and width of the trabeculae on the horizontal lines crossing the vertebral body were measured. $\times 3$.

Fig. 3B. Microradiograms at four representative areas of vertebral body. $\times 40$.

Note: *a* and *b* represent the same sites for both figures.

Fig. 3 B

treated as controls for statistic study. Others included 45 cases of cancer, 8 of liver cirrhosis, 3 of renal insufficiency, 8 of sarcoma, and 5 of pulmonary tuberculosis. The obtained values were statistically analyzed in reference to each group of diseases and of ages. The number of sample, arithmetic mean and unbiased variance are represented by the letters of N, $\bar{x}$ and u^2 , respectively, as shown in tables.

RESULTS

1) *Distribution of mineral in the vertebral body*

The difference in the grade of mineralization of vertebral body is surveyed in a number of representative specific areas, namely the rim of the vertebral body, the calcified fibrous cartilage on the rim, the end plate of vertebral body, the calcified hyaline cartilage adjacent to the end plate, the trabecula, and the cortex of the vertebral body. In the area in which the haversian system exists, measurement was made on the interstitial zone (Fig. 3). No significant difference between calcified fibrous cartilage on the rim and calcified hyaline cartilage was found throughout

TABLE 1. *The grade of mineralization at the calcified fibrous cartilage on the rim of the vertebral body and the calcified hyaline cartilage adjacent to the end plate*

	Decade of age	N	$\bar{x}$	u^2
Calcified hyaline cartilage adjacent to end plate	2	8	143	92
	3	6	127	238
	4	7	122	131
	5	15	124	541
	6	15	132	166
	7	11	126	393
	8	10	139	379
Calcified fibrous cartilage on the rim of vertebral body	2	8	144	155
	3	6	128	601
	4	7	125	350
	5	15	128	480
	6	15	134	205
	7	11	126	242
	8	10	147	36

N: Number of samples

$\bar{x}$: Arithmetic mean

u^2 : Unbiased variance

the investigation (Table 1). Therefore, they were included in the same category as calcified margin of the vertebral body. The values at the rim of vertebral body were found to be almost the same to that at the end plate. Therefore, these were represented by measurements on the end plate of vertebral body. On the average of all cases the calcified margin of vertebral body was found to show the highest value, and the vertebral end plate and trabeculae at the spongy portion exhibited the second highest rate and then followed by cortical bone.

2) *The change of mineralization rate by age in control group*

In the control group the grade of mineralization in the calcified margin of the vertebral body in the fetal age to the first decade was represented by that in the calcified cartilage and the non-remodeled portion of the trabeculae; and in the trabeculae, by that in the remodeled portion of the trabeculae.

The values of mineralization rate at calcified margin of the vertebral body in the second to eighth decades were from 128 to 143 and those in 7 months of fetal age to the first decade were from 170 to 167, which were obviously larger than the former. The end plate and the trabeculae revealed nearly the same value of bone salt content and they had a similar tendency in changes of mineralization by age.

TABLE 2. *The grade of mineralization in four representative areas of vertebral body*

	Decade of age	N	$\bar{x}$	u^2
Calcified margin of vertebral body	Fetus	4	170	7
	1	3	167	73
	2	2	143	67
	3	5	134	73
	4	0		
	5	4	128	45
	6	5	129	55
	7	4	141	226
	8	5	138	55
End plate	Fetus			
	1			
	2	2	83	130
	3	5	111	105
	4	0		
	5	4	116	12
	6	5	101	31
	7	4	113	87
	8	3	120	12
Trabeculae	Fetus	4	130	107
	1	3	133	83
	2	2	93	20
	3	4	114	122
	4	0		
	5	4	114	16
	6	5	104	56
	7	4	112	19
	8	3	123	12
Cortex	Fetus			
	1			
	2	2	73	126
	3	3	95	7
	4	0		
	5	4	98	78
	6	5	92	73
	7	4	103	117
	8	4	101	135

In fetus and in the first decade of age, the values at trabeculae were found to be from 130 to 133, whereas those at trabeculae and vertebral end plate in the 2nd decade of life were as low as 95 to 83. It might be due to that the vertebral end plate and remodeled trabeculae are differentiated from the cartilage after the second decade of age and to elevated remodeling activity of bone tissue. After the cease in growth of skeletal system, the values at vertebral end plate and trabeculae were 101 to 120 and 104 to 123, respectively, without any further significant change through the life. The cortex of the vertebral body, was found to have values from 73 to 103, which were the lowest values among the three parts of the vertebral body (Table 2). As described above, only a small fluctuation was observed in the grade of mineralization in the four parts of the vertebral body after the third decade of life. These mean values are shown in Table 3.

TABLE 3. *The mean value of the grade of mineralization in four areas of the vertebral body over the third decade of age*

	N	$\bar{x}$	u^2
Calcified margin of vertebral body	21	134	80
End plate	21	111	89
Trabeculae	20	112	83
Ccrtex	20	94	79

3) *The mineralization of vertebral body in pathological conditions*

The vertebral bodies obtained from six patients who died of lung cancer with no metastasis to the spine were found to have obviously larger amount of mineral content in all the portions of vertebral body than in the control. On the other hand, no changes were found in those who died of cancer of the digestive tract or the maxillary bone (Table 4). In each of hypernephroma, malignant chorionepithelioma, cancer of the prostate associated with bile duct involvement, and cancer of the urinary bladder, the values were found to differ from one another, although they were generally higher than in the control (Table 5). In 8 cases of sarcoma, there was no noticeable difference (Table 6). The grade of mineralization of vertebral body in 8 cases of liver cirrhosis was found to be low. On the contrary, in 3 cases of renal insufficiency it was considerably high in all the examined portions of vertebral body except in the calcified margin. No difference was found in cases of pulmonary tuberculosis (Table 7).

4) *Trabecular density*

The number and width of trabeculae in medullary portion was surveyed in the cases over the third decade of age as shown in Table 8. The number of intersecting trabeculae on a unit length showed its maximum as high as 7.4 per cm

TABLE 4. *The grade of mineralization in the vertebral body in various carcinomas without vertebral metastasis*

	Parts of vertebral body	N	$\bar{x}$	u^2
Lung cancer	Calcified margin of vertebral body	7	150	121
	End plate	7	124	103
	Trabeculae	7	128	88
	Cortex	7	117	99
Cancer of the maxillary bone	Calcified margin of vertebral body	6	130	515
	End plate	6	105	287
	Trabeculae	6	105	80
	Cortex	6	95	326
Stomach cancer	Calcified margin of vertebral body	7	134	62
	End plate	9	109	267
	Trabeculae	9	109	198
	Cortex	9	97	296
Carcinomas in the digestive canal except for the stomach	Calcified margin of vertebral body	7	122	338
	End plate	7	105	381
	Trabeculae	7	107	302
	Cortex	7	99	369

TABLE 5. *High grade of mineralization in cases of malignant tumors*

	Calcified margin of vertebral body	End plate	Trabeculae	Cortex
Hypernephroma	146	123	129	110
Malignant chorionepithelioma	143	124	127	120
Cancer of the prostate associated with the bile duct	140	131	131	109
Cancer of the urinary bladder	161	136	137	125

TABLE 6. *The grade of mineralization in cases of sarcomas*

	N	$\bar{x}$	u^2
Calcified margin of vertebral body	8	131	180
End plate	8	107	178
Trabeculae	8	107	261
Cortex	7	92	297

TABLE 7. *The grade of mineralization in the vertebral bodies in various disorders*

	Parts of the vertebral body	N	$\bar{x}$	u^2
Liver cirrhosis	Calcified margin of vertebral body	8	111	26
	End plate	8	89	86
	Trabeculae	7	93	66
	Cortex	8	82	157
Renal insufficiency	Calcified margin of vertebral body	3	143	163
	End plate	3	131	31
	Trabeculae	3	131	18
	Cortex	2	116	8
Pulmonary tuberculosis	Calcified margin of vertebral body	4	137	148
	End plate	4	112	59
	Trabeculae	4	105	77
	Cortex	4	101	82

TABLE 8. *The number and width of trabeculae in reference to age*

Decade of age	N	Number of trabecula (cm^{-1})		Summation of the width of trabeculae (mm/cm)		Average width of trabeculae (mm)	
		$\bar{x}$	u^2	$\bar{x}$	u^2	$\bar{x}$	u^2
3	15	7.4	29.3	1.05	7.12	0.13	5.11
4	15	7.0	17.0	0.79	5.56	0.11	5.99
5	21	6.5	33.1	0.70	5.95	0.11	8.77
6	21	5.0	28.5	0.55	3.38	0.11	3.39
7	24	4.8	17.4	0.53	2.97	0.11	3.26
8	14	4.2	26.1	0.49	7.30	0.10	7.53

in the third decade, then it gradually decreased with advancing age to as low as 4.2 in the eighth decade of life. In this process of decrease, the diminution was remarkable from the fifth to sixth decade. The width of each trabecular per cm of length in vertebral body was measured and summed up; it was 1.05 mm/cm in the third decade, and 0.49 mm/cm in the eighth decade, with a notable lowering in its value between the fifth and sixth decades. The pattern of decrease in trabecular width by age resembled that in the trabecular number by age. The average width of the trabeculae was 0.13 mm in the third decade, without any notable change thereafter.

The number and width of the trabeculae showed on the whole similar values in both sexes, although the females over the seventh decade were apt to have considerably low values. There was no difference in the average width of the

TABLE 9. *The number and width of trabeculae against sex difference*

Decade of age	Sex	N	Number of trabeculae (cm ⁻¹)		Summation of the width of trabeculae (mm/cm)		Average of width trabeculae (mm)	
			$\bar{x}$	u^2	$\bar{x}$	u^2	$\bar{x}$	u^2
3	♂	14	7.4	26.8	1.08	7.24	0.13	6.07
	♀	3	7.4	20.1	0.93	7.47	0.13	0.61
4	♂	6	7.5	16.2	0.78	7.87	0.11	7.10
	♀	9	6.6	1.77	0.79	4.65	0.11	5.81
5	♂	16	6.5	30.6	0.70	5.66	0.11	10.81
	♀	5	6.3	29.0	0.71	4.62	0.11	2.96
6	♂	14	5.0	30.8	0.55	3.72	0.11	2.93
	♀	7	5.1	28.0	0.56	3.73	0.11	4.32
7	♂	19	4.9	16.7	0.56	2.92	0.11	3.08
	♀	5	4.1	17.7	0.42	2.17	0.11	1.79
8	♂	10	4.7	16.5	0.57	7.37	0.11	9.52
	♀	4	3.1	41.3	0.37	0.51	0.09	0.12

trabeculae between both sexes (Table 9).

DISCUSSION

Quantitative measurement of calcium in bone tissue has been done previously by means of biochemical techniques, in which calcium salt is measured on a certain weight or volume of bone pieces. It is impossible, however, to know the quantitative distribution of minerals in direct relation to the histological findings of bone tissue by this method. Since quantitative microradiography was applied to the study of bone structure by Engström¹ in 1946, the modes of mineralization have been investigated in different conditions of the bone by many investigators.

In this method, the quantity of bone mineral is expressed by the value of thickness of standardized material or by the value of absorption coefficient derived from the degrees of blackness of the processed film after its exposure to roentgen ray through a tested bone. The calcium salt in bone tissue is thought to be hydroxyapatite and this is the main substance related to absorption of roentgen ray in bone tissue. From the values of linear absorption coefficient obtained from the present experiments and the mass absorption coefficient of hydroxyapatite, it is possible to know the density of hydroxyapatite in bone tissue *in situ*, consequently the quantity of Ca deposition in the bone at different areas can be estimated. According to Engström⁴ the mass absorption coefficient of hydroxyapatite is given by $\mu/\rho = 83 \cdot \text{g}^{-1} \cdot \text{cm}^2$, where μ is the absorption coefficient per cm and ρ is the density in $\text{g} \cdot \text{cm}^{-3}$. The values obtained from the normal vertebral bodies are shown in Table 10. These are found to be consistent with those by Sissons *et al.*⁷ who employed the methods of Alwedge. The density of hydroxyapatite in the vertebral bodies at embryonic stage and until 9 months of life was found to be 2.04, a value very close to 2.0 of pure hydroxyapatite. It

TABLE 10. *The linear absorption coefficient of vertebral bodies over the 3rd decade and its relation to the quantity of hydroxyapatite and calcium*

	Linear absorption coefficient $\mu(\text{cm}^{-1})$	Amount of hydroxy-apatite (g/cm^3)	Weight of calcium (g/cm)
Calcified margin of vertebral body	134	1.61	0.64
End plate	111	1.34	0.54
Trabeculae	112	1.35	0.54
Cortex	94	1.13	0.45

is assumed from this finding that the maximum density of bone salt is found in the area where bone salt deposition takes place in early ages, provided that the bone salt in early ages is hydroxyapatite. The determination of composition of bone salt in such an early age is beyond the scope of the present observation.

Sissons *et al.*⁷ showed on cross section specimens of the femur that the haversian canals became widened and the ratio of highly calcified osteon was increased with the advance of age. On the other hand, Jowsey *et al.*¹⁰ reported a lower ratio of calcified osteons in the femur with osteoporosis as well as Bohr,¹² who obtained similar results from pieces of iliac bone in osteoporosis.

It was found in the present investigation that there was always a definite order in the distribution of bone salt in respective portions of vertebral body. The grade of mineralization was shown in the following order from high to low grade: The calcified margin of vertebral body, end plate of vertebral body or bone trabeculae in the spongy marrow, and cortical bone. The calcified marginal area is presumably not remodeled. Accordingly, its bone salt content was found to become decreased gradually with the advance of age, whereas the bone salt content in bone trabeculae, end plate of vertebrae and cortex in the second decade of age were found to be remarkably decreased. This would be interpreted to be due to an active remodeling of skeletal tissue in this decade. After the third decade of age, the bone salt content did not change significantly. The dynamic strongness of the bone, which is larger in the third decade than in any other age of life, is not necessarily due to a large bone salt content, and is thought to be more closely related to the mode of arrangement of bone trabeculae and their density in skeletal tissue.

The summation of the widths of respective bone trabeculae on a unit length has a parallel relation to either the number or the average width of bone trabeculae, consequently to the trabecular density (Table 11). It was found in the present investigation that the density of bone trabeculae had its maximum in the third decade of life. Then it became decreased with advancing ages, particularly in the fifth and sixth decades of life. As mentioned above, the dynamic strength of bone is largest in the third decade where the density of trabecula has its maximum value. The reports of Bauer¹³ and Tatematu¹⁴ that the incidence of pathological fracture

TABLE 11. *The number of trabeculae, summation of their widths and average width of trabecula*

Decade of age	N	Correlation		
		Number and summation of widths of trabeculae	Summation of width of trabeculae and average trabecular width	Number and average width of trabeculae
3	15	0.55	0.59	(-)
4	15	(-)	0.83	(-)
5	21	0.49	0.44	(-)
6	21	0.91	(-)	(-)
7	24	0.75	0.46	(-)
8	14	0.31	0.59	(-)

(-): No significant relation.

is most frequent in the fifth decade may be explained by the result of the present study. The condition of the bone in this age characterized by decrease in the trabecular density and predisposing pathological fractures is to be designated as osteoporosis.

The vertebral bodies obtained from patients who died of lung cancer, hypernephroma, prostatic cancer and cancer of urinary bladder without metastasis to the spines showed evidently high mineralization. It is widely known that cancers of breast, lung and prostate or hypernephroma very frequently metastasize to skeletal tissue. As one of the causes¹⁵⁻¹⁹ contributing to this process, hypercalcemia has been generally taken into consideration.^{2,5,6,13,16} However, Ehrlich²⁰ observed hypocalcemia, hypoparathyroidism or elevated rate of bone formation in a case of prostatic cancer accompanied by osteoplastic metastasis to the bone tissue. In our four cases of cancer in which serum Ca was examined, no hypercalcemia was found, whereas hypocalcemia was noted in a case of urinary bladder cancer.

Of four cases of renal insufficiency in the present series of observation, three cases apparently showed high Ca content and the other showed, on the contrary, a low level. The serum Ca level was apt to be rather low in the cases of high Ca content in the bone. The tendency of low calcium level in serum should be taken in consideration for high Ca deposition in the bone.

Atkinson (1956)²¹ observed osteoporosis or osteomalacia in 7 cases out of 25 of hepatic and post-hepatic jaundice. In the present investigation, all the 8 cases of liver cirrhosis showed remarkably low grades of bone salt content. Atkinson also pointed out that the change in skeletal tissue was noticed within 6 months after the beginning of hepatic jaundice, but it appeared evident only after more than 3 years in the cases of post-hepatic jaundice. In the present investigation, similarly, the cases of post-hepatic jaundice had not any abnormality in mineralization of vertebral bodies 4 months in one case, and one year and 8 months in another case after the onset of the disease. Presumably, the decrease in bone salt content is due to vitamin D deficiency induced by functional disturbances of liver

parenchym as pointed out by Atkinson, or insufficiency in the formation of ground substance in the bone. The application of quantitative microradiography in the present investigation for determining the grade of mineralization in the bone must be further extended to the bone in pathological conditions and for a diagnostic device combined with biochemical study with radioisotope and with the study of dynamic hardness of bone with an osteosclerometer.

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