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SCANNING ELECTRON MICROSCOPIC STUDY OF THE HUMAN ADULT BONES FOR DETERMINING THE INDIVIDUAL AGE

ABSTRACT: Scanning electron microscopic studies were carried out to examine the finer structure of the grown-up bones due to age. Bone samples from the middle part of the femur of 25 individuals of different age (13 – 78 years) from our obduction material were studied. The studies were carried out by the electron microscope type TESLA BS 300 at 25 kV accelerating voltage. The scanning electron microscopic study is a suitable method to determine the individual age in the forensic medical practice and historical anthropological research on the basis of studying the bone structure. According to our studies it is possible to determine the age not only by the "quantitative" method of Kerley (1965) but also on the basis of the "qualitative" structural study by considering the aspects showing the alterations and changes of bone structure. The failure in determining the age by the latter method cannot exceed 10 years.

KEY WORDS: Human adult bones – Scanning electron microscopic study – Individual age determination – Application in forensic and historical anthropology.

INTRODUCTION

The knowledge about the group characteristics of the human bones and the possibility to differentiate them from animal bones is important from forensic medical aspects (Ahlquist and Danisten 1969, Cook 1961, Krogman 1939, McKern et al. 1957, Schranz 1959, Stewart 1954). The intensive research of the bone structure started at the beginning of the century, first on thin bone sections and later on decalcified histological segments (Amprino 1965, Amprino and Bairati 1936, Bocciarelli 1970, Brown 1966, Budy 1968, Eanes et al. 1966, Fourman 1960, Földes et al. 1980, Holmstrand 1957, Höhling 1969, Kósa et al. 1980, Kramer and Shear 1928, Lengyel 1968, 1973, Neuman and Neuman 1958). On the basis of this research, sufficiently elaborated methods are available to determine the individual age on the basis of bone structure by light microscopic studies (Amprino and Maroti 1964, Jowsey 1960, 1964, 1966, Kerley 1965, 1969). Mainly the method of Kerley (1965) is applied in forensic medical practice and historical anthropological research. He regarded the 'maturity process' of the osteon structure of the bones at determining the age on the basis of the frequency and interrelation of the new osteons, resorptive osteons, matrix and „Non – Haversian" canals (the so called Volkmann canals) nourishing the bones (Cohen and Harris 1958, Currey 1964, Enlow 1962, Frost 1963, Lacroix 1951, Ortner 1967, Oakley 1955, Posner 1969, Robinson et al. 1955, Shear and Kramer 1928, Strand 1960, Termine 1966, Zambotti and Bolognani 1967). The individual age of the bones can be estimated by good approximation to 5 – 7 years (by the regression linears of Kerley, 1965).

The regulations of the age group changes in the cortex of the extremital bones were explored by Amprino and Bairati (1936) and Jowsey (1960), respectively. The activity in the extremital bones is the most intensive in the period from birth till the 3rd year. It happens in the medullar wall of cortex and includes the whole width of cortex at the age of 4 – 11. The

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intensive resorption phenomenon can be observed below periosteum in the outer third at the age of 10 – 17. Following the 17th year, the activity ceases on the outer area of cortex and a homogeneous bone structure develops gradually. Later it is followed by the resorptive activity of the medullar wall. This phenomenon can be observed in women at the beginning of their 40's. However, it never can be observed in men before the 40th year.

Kerley (1965) refers exactly to the qualitative and quantitative aspects which must be considered for studying the bone structure at determining the age. They were used in our SEM studies.

- The osseous structure always stretches from the direction of the medullar wall to the direction of the outer edge in case of the long tubular bones.
- The number of fragmental osteons increases with age.
- The thickness of the bones grows because the osteoblasts form a fine lamellar layer below the periostem similarly to the lamellae of the union.
- This outer circular lamellar layer contains vertically running blood vessels remaining from periosteum. They become to the so called Volkmann canals or „Non – Haversian" canals. In a later period of the osseous development osteons are formed around them, too.

When estimating the number of osteons, those are considered intact which are regular in 80 % and have a sound shape.

According to Kerley (1965), the following regression equations can be used in determining the age on the basis of the structure of extremital bones:

Studied cortex	Regression	Correlation	Scattering
	FEMUR		
Regular osteons	$y = 3.473 + 0.144X + 0.003X^2$	$p = 0.922$	±9.39
Fragmental osteons	$y = 8.786 + 0.834X$	$r = 0.864$	±12.19
Lamellar bones	$y = 79.455 - 2.427X + 0.023X^2$	$p = 0.870$	±11.78
"Non-Haversian" canals	$y = 57.811 - 1.728X + 0.013X^2$	$p = 0.815$	±13.85
	TIBIA		
Regular osteons	$y = 10.082 + 0.634X$	$r = 0.925$	±6.69
Fragmental osteons	$y = 7.061 + 0.931X + 2.210X^2 - 2.538X^3$	$p = 0.947$	±7.78
Lamellar bones	$y = 76.338 - 1.794X + 0.794X^2$	$p = 0.816$	±13.62
"Non-Haversian" canals	$y = 70.270 - 10.944X + 0.647X^2 - 0.011X^3$	$p = 0.790$	±9.63
	FIBULA		
Regular osteons	$y = 2.366 - 0.538X + 0.018X^2 - 0.001X^3$	$p = 0.922$	±8.83
Fragmental osteons	$y = 1.328 - 0.058X + 0.034X^2$	$p = 0.974$	±5.27
Lamellar bones	$y = 69.108 - 2.208X + 0.015X^2$	$p = 0.881$	±10.85
"Non-Haversian" canals	$y = 55,241 - 4,300X + 0,050X^2$	$p = 0,879$	±10,70

The scanning electron microscopic and electron microprobe studies have recently become more and more important in the forensic and historical studies (Amprino and Engström 1952, Baud and Morgenthaler 1952, Bergman and Engfeldt 1934, Birks 1963, Boyde et al. 1962, Carlström 1957, Cooper et al. 1966, Cosslett 1962, Engström 1960, Mellors et al. 1964, Pellegrino and Blitz 1968, Wallgren 1957). By using this method, difficulties like making bone sections by much and difficult work or decalcification of bones can be obviated. Damages of the bone samples can also be prevented which made the metrical evaluation difficult for determining the dimension of osteons and the diameter of Haversian canals in determining the age or in differentiating human bones from animal bones. Our objective was to elaborate the scanning electron microscopic study of bones by which the individual age can be determined. There has been osteological study in our institute for years and we have had the possibility to carry out SEM studies. A further advantage of the SEM studies is that the study of the bones can be carried out practically immediately without any circumstantial preparation of bones in laboratory if the necessary equipment is available. The result might be received within an hour, and this fact is essential from the forensic aspect.

MATERIALS AND METHODS

Scanning electron microscopic studies were carried out to study the finer structure of the bone substance due to age.

Bone samples from the middle part of the femur of 25 individuals of different age (13 – 78 year-old) from our obduction material were studied.

Bone samples were defatted and dehydrated in a mixture of ether and aceton and dried in an incubator at 37°C. The structure of bones was studied on the transverse fracture surface. The bone fragments were fixed on the preparate holding plates by Colloida Silver (Polaron LTD, Watford, England) adhesive. The bone surface was coated by 300 Å golden layer in the vacuum evaporator type Polaron SEM Coating Unit E 5100 at a vacuum of 0.02 tor and 20 mA intensity of current. SEM studies were carried out by the electron microscope type TESLA BS 300 at 25 kV accelerating voltage.

RESULTS AND DISCUSSIONS

On the basis of the data from recent literature the aspect has already been accepted that the tubular bones rebuild and their structure renews during life-time.

The process of change of the bone structure shows the actual age of the individual. Our data show as well that these age group characteristics have at least as much regularity as the annual rings of the trees, from which the age of the tree can be estimated by high certainty.

In case of bones the equation has not one unknown but many of them. The problem is more complicated and essentially more aspects must be considered at estimating age.

In our opinion, not only the correlations of Kerley (1965) are valid and essential, which represent the correlation of bone structure and age, but numerous other traits like the dimension of osteons, the number of the circular lamellae of the osteons, the ratio between the circular and semicircular lamellae, the distance between Haversian canals, etc.

These correlations will be clarified and analysed in systematical studies by scanning electron microscopic pictures.

Our present report has two objectives:

- To elaborate the method of scanning electron microscopic study of bones, which is suitable to study the bones for determining the age in the forensic medical and historical anthropological research.
- To reanalyse the method of Kerley (1965): how accurate are his aspects in the actual age determination on the basis of study of bone structures. The age group changes observable in the bones of the individuals of different age by scanning electron microscopy can be characterized as follows:

Even an overall view of the bones provides valuable information about the individual age for an experienced expert who has already studied many bone cross-sections either by traditional histological or scanning electron microscopic method.

First of all several scanning electron microscopic pictures are shown of the bones of individuals of different age to verify the above statement.

Figure 1. The outer zone of the femur of a 13 year-old boy (No. 101, SEM 100×).

Mainly lamellar bone tissue can be seen with the cross-section of vascular canals, around which no osteons with lamellar structure occur.

Figure 1.

Figure 2. A picture of the middle part of the fracture surface of the above femur. Highly magnified (No. 100, SEM 1000×).

Two osteons can be seen which have already a lamellar structure, but only a lamellar layer consisting of several rows can be guessed around the Haversian canals. It can also be stated that there is a matrix between

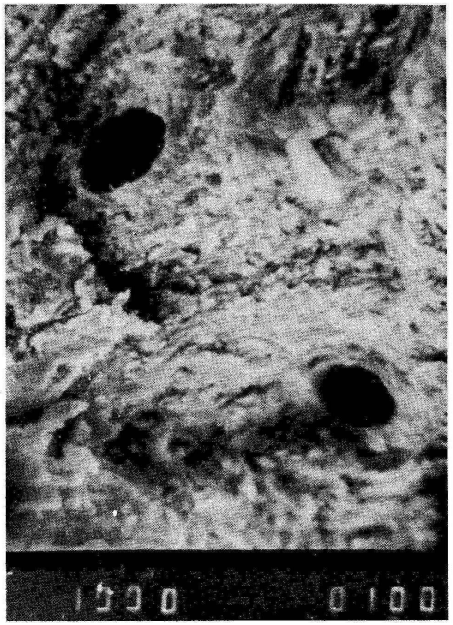

Figure 2.

the osteons which is at least as wide as a Haversian canal, and which looks like a lamellar bone. There are no resorptive osteons or they are hardly observable. So childhood and young adult age is characterized by few, well-separately situated osteons consisting of few circular lamellae. At this age there are no multilayered osteons.

Figure 3. A picture of the transverse fracture surface of the femur of a 23 year-old man (No. 112, SEM 100×).

Practically separately situated osteons can be found. Their outer edges may communicate but resorptive osteons (*laminae intercalares)* are not characteristic for this age.

Figure 4. The same picture enlarged (No. 119, SEM 500×). The larger osteon found on the fracture surface consists of 13, the smaller one of 9 circular lamellae. They communicate, moreover, the larger osteon has already absorbed the lamellae of the smaller one in part. It increased on the smaller one's expense.

Figure 5 shows the 'largest' regular osteon found in the femur of a 35 year-old man (No. 110, SEM 500×). The number of lamellae is above 13. The diameter of the osteon is 100 microns, that is the double of the osteon of the 23 year-old man in *Figure 4*. (The dimension of the smaller osteon in *Figure 4* is 50, while that of the larger one 80 microns!).

Figure 6. This picture represents the fracture surface of the femur of a 58 year-old man (No. 104, SEM 300×).

Figure 3.

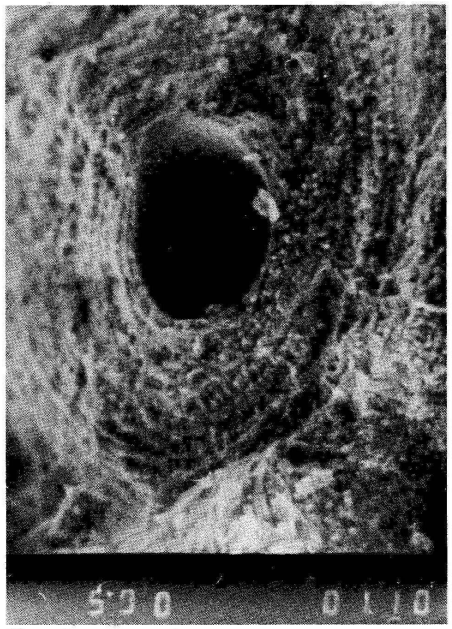

Figure 5.

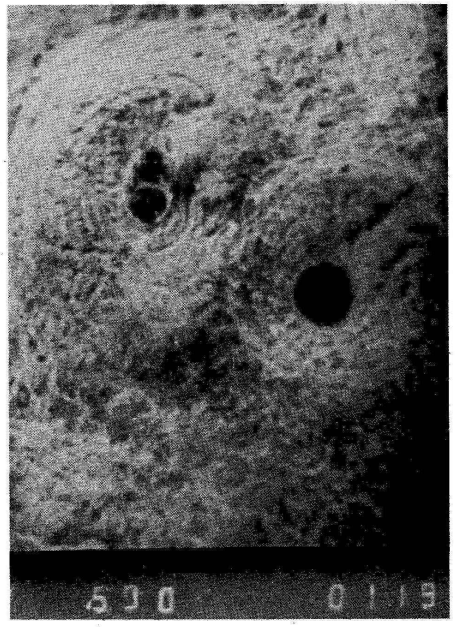

Figure 4.

Figure 6.

Osteons of resorptive character can mainly be seen. On the top there is a larger osteon. The number of the circular lamellae in it is above 13.

Figure 7. An enlarged picture of the osteon from the previous picture (No. 107, SEM 500×). The number of the circular lamellae of the osteon is 15 and its diameter is 90 microns.

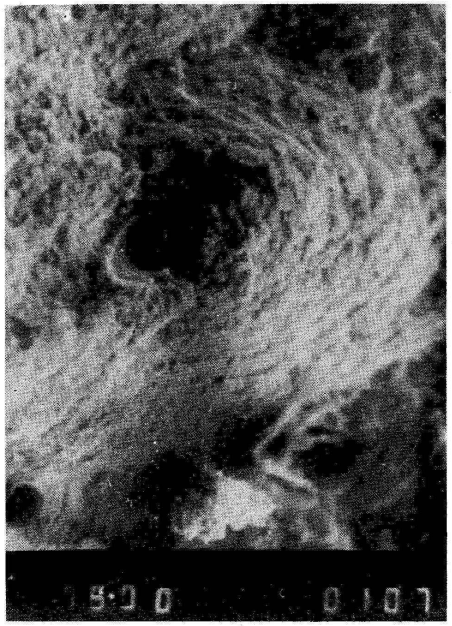

Figure 7.

Figure 8. A picture of the fracture surface of a 60 year-old man (No. 6658, SEM 700×). A regular osteon in which the number of the circular lamellae is 15. That was the highest number of lamellae found in the osteons. The conclusion has been drawn that this is the osteon structure of the highest dimension which can be in equilibrium with the homeostasis of the organism for a time, but later it will be resorped by a recently developed osteon.

Two SEM pictures will be shown now. In one of them *(Figure 9)* the typical bone structure of a 23 year-old young individual can be seen (No. 118, SEM 100×). The bone consists of regular round osteons with small diameter situated farther from each other in the matrix. There are no 'ruin – osteons', i.e. resorptive osteons. The diameter of the osteons is generally 60 microns.

In *Figure 10* on the other hand, is the bone structure of a typical aged individual (72 year-old) (No. 6655, SEM 300×). The view is very heterogeneous. The majority of the osteons are secondary osteons (*laminae intercalares*). Besides them, osteons of very small dimensions can be observed consisting of few circular lamellae.

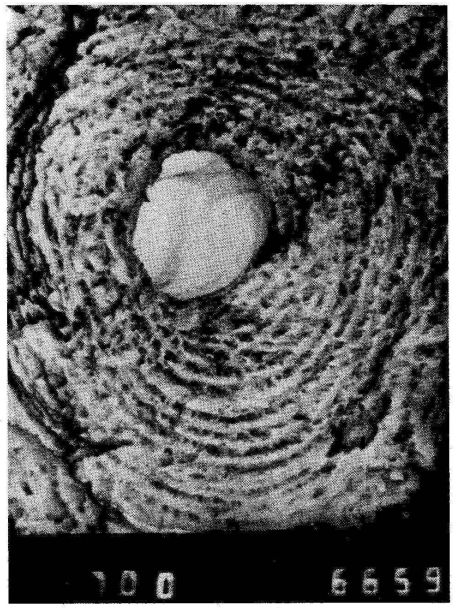

Figure 8.

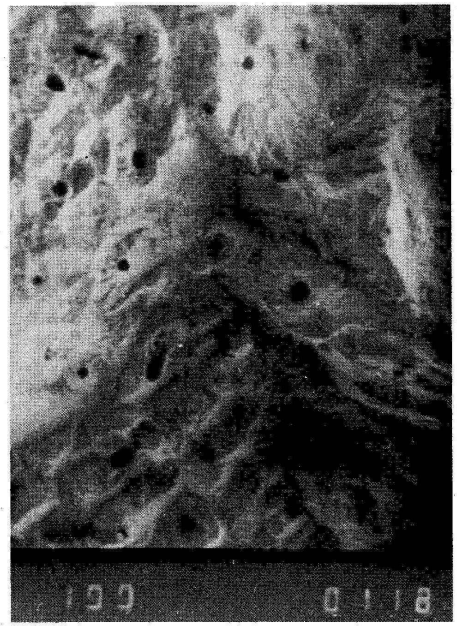

Figure 9.

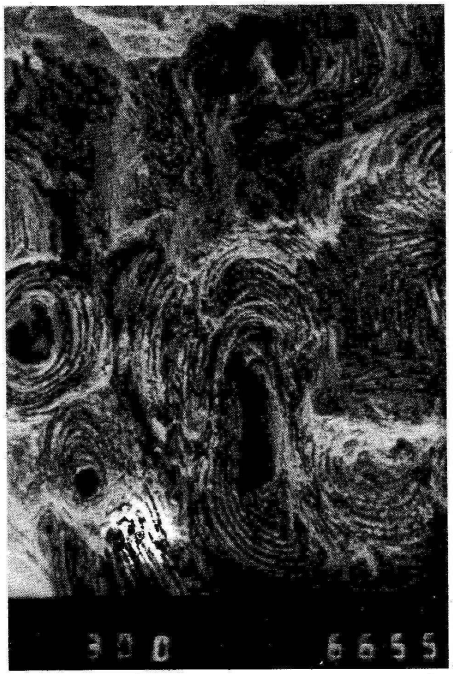

Figure 10.

In *Table 1* the data of the bone sample from our material are demonstrated. The age is indicated by the Kerley method and a qualitative estimation compared to the actual age.

Our studies show that SEM study of the bones gives a possibility to determine the individual age by considering the structure of the bones. The age of the adult individuals can be estimated by 10 years difference not only by the method of Kerley (1965) but also by regarding the morphological characteristics of the bones and considering the qualitative traits. This is the error limit which generally occurs in similar size in evaluating biological phenomena.

Table 1. Comparative study of age by the method of Kerley (1965) and on the basis of the qualitative SEM study of the tubular bones.

Age, sex	Age determined on the basis of qualitative changes	Difference (± year)	Age determined on the basis of quantitative changes (Kerley method)	Difference (± year)
1. 13 ♂	15	+2	15	+2
2. 18 ♂	20	+2	22	+4
3. 21 ♀	20	−1	27	+6
4. 22 ♀	20	−2	25	+3
5. 23 ♂	30	+7	25	+2
6. 29 ♀	30	+1	30	+1
7. 32 ♀	30	−2	35	+3
8. 35 ♂	40	+5	35	0
9. 30 ♂	40	+2	34	−4
10. 42 ♂	40	−2	38	−4
11. 45 ♂	50	+5	40	−5
12. 48 ♂	50	+2	45	−3
13. 52 ♀	50	−2	50	−2
14. 54 ♀	60	+6	50	−4
15. 57 ♂	60	+3	51	−6
16. 58 ♀	60	+2	52	−6
17. 63 ♀	60	−3	55	−8
18. 65 ♂	70	+5	58	−7
19. 69 ♂	70	+1	60	−9
20. 71 ♂	70	−1	75	+4
21. 73 ♀	70	−3	78	+5
22. 75 ♀	70	−5	78	+3
23. 77 ♀	70	−7	85	+8
24. 79 ♀	80	+1	82	+3
25. 87 ♀	80	−7	85	−2

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