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OBSERVATION OF THE MICROSTRUCTURE OF RAT CORTICAL BONE TISSUE

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Abstract

The detailed microscopic structure (qualitative and quantitative characteristics) of rat (female) cortical bone tissue is reported. *Femur* diaphysis from each individual was sectioned at its smallest breadth. The obtained segments were embedded and consequently cut by a sawing microtome (Leitz 1600) to the final thickness of 100 microns. The average areas, perimeters, minimal and maximal diameters of 100 vascular canals of primary osteons were measured using the digital image device. According to our study the investigated cortical bone tissue is in general non–vascular. The tissue is composed solely of concentric cellular lamellae, vascular canals (primary or secondary) are absent. There are some areas of primary vascular radial bone tissue identified in anteromedial sides and areas of primary vascular reticular bone tissue in the middle of radial compacta. Haversian bone tissue is not formed. The vascular canals of primary osteons measured are very short.

Key words

Rat, Femur diaphysis, Microstructure, Histomorphometry

INTRODUCTION

Bone is a living connective tissue which remodels a lifetime. The principal cells which mediate its structural and functional properties are: osteoblasts, osteocytes, and osteoclasts (*2*). According to the *Wolff's* (*10*) law, different animal species with specific phylogenetic and life histories should exhibit different bone microstructure as a result of adaptation to species–specific claims.

The wall of adult rat long bone diaphysis is built of compact bone tissue. Basic constituents of its structural organization are primary and secondary (Haversian) osteons. The primary osteon is a vascular canal that is not surrounded by Haversian lamellae and does not contain a cement line (*4*), the secondary one is composed of elementary structures like concentric lamellae, Haversian canal, lacunae, and canaliculi (*1*). Histological research of the microstructure of compact bone tissue can be carried out in two ways: qualitatively and quantitatively *(6).* Regarding the

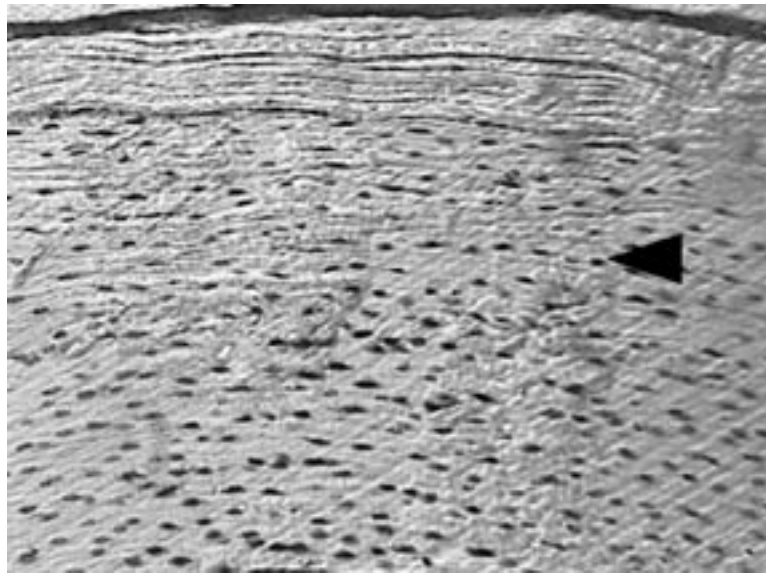

Fig. 1
Non-vascular bone tissue composed of concentric cellular lamellae (arrow; magnification x 200)

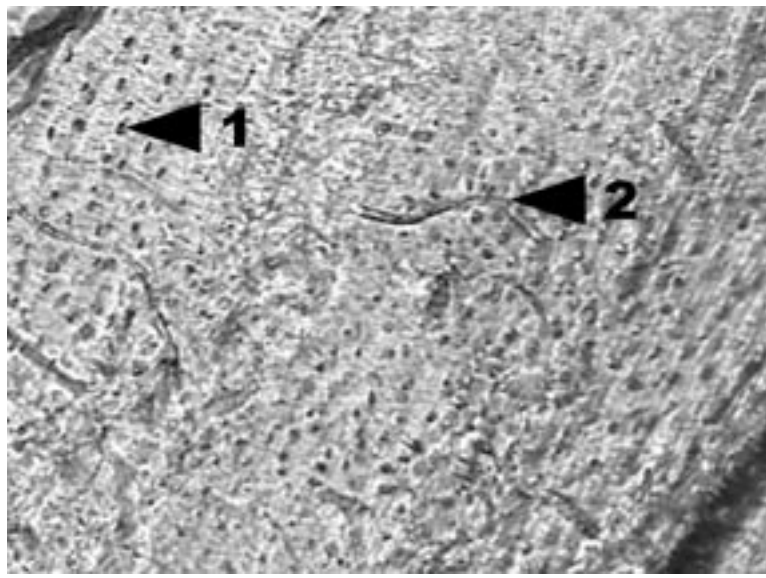

Fig. 2
Primary vascular radial bone tissue (magnification x 200). 1 – osteocytes; 2 – vascular canals

Table 1
Basic statistical characteristics of vascular canals of primary osteons

Measured structures	n	Parameters	x	s	v	med.	min.	max.
Vascular canals of primary osteons	100	area (μm²)	83.97	25.61	30.49	78.04	37.04	185.19
		perimeter (μm)	26.50	5.28	19.92	25.88	15.10	41.89
		max. diameter (μm)	13.12	3.76	28.65	12.28	6.85	21.46
		min. diameter (μm)	4.19	1.07	25.54	3.87	2.17	6.99

x – average, s – standard deviation, v – coefficient of variance, med. – median, min. – minimum, max. – maximum

quantitative approach, counts and measures of the basic structural characteristic can be made (e. g. diameter of primary osteons' vascular canals, area of secondary osteons); considering the qualitative approach, the structural pattern of compact bone tissue is identified.

The goal of this study was to analyse the microstructure of rat *femur* diaphysis. Microscopic structure of the cortical bone was evaluated from the point of view of qualitative and quantitative characteristics.

MATERIAL AND METHODS

Our research focused on 8 *femurs* of four 5–6 months old female rats of Wistar breed. Each of the bones was sectioned at the smallest breadth of its diaphysis where the compact bone is thick and provides a large area for study of the bone tissue microstructure. In total, 8 transversal sections of the *femur* diaphysis were cut. The obtained segments were macerated and degreased. Later the samples were embedded in Biodur epoxide resin (Gunter von Hagens). Transverse thin sections (100 μm) were prepared with a sawing microtome (Leitz 1600) and stuck on glass slides with Eukitt.

For the examination, an optical microscope Jenaval (Carl Zeiss Jena) with a digital CCD camera (Mintrow) at a magnification of 200x were used. Photographic documentation of the slides was made using computer programs Ati Player 5.2 (Ati Technol. Inc.) and Adobe Photoshop 5.0. The qualitative characteristics were determined according to the generally known and internationally accepted classifications by *Enlow and Brown* (*3*), *Rämsch and Zerndt (9),* and *Gladuhsew (5).* The quantitative characteristics were found out using the computer software Scion Image (Scion Corporation, USA). We measured the following parameters: area, perimeter, minimal and maximal diameter of 100 primary vascular canals of primary osteons. The basic statistical characteristics of the position and variability for each of the particular parameters were counted using the Statistica 4.3 software package.

RESULTS

The *femurs* of all the analysed animals had the following microstructure in common. The arrangement and distribution of different bone tissue types is given from the medullary cavity towards the periosteal surface:

– the inner layer surrounding the medullary cavity is formed by a zone of non–vascular bone tissue (*Fig. 1*) which contains concentric cellular lamellae and osteocytes.

Vascular canals, primary or secondary, are absent. There are some areas of primary vascular radial bone tissue identified in anteromedial sides (*Fig. 2*). The tissue is composed of branching or unbranching vascular canals which radiate from the marrow cavity. In these parts there are a few resorption cavities too. They testify to intensive bone tissue remodelling. However, Haversian bone tissue is not formed.

– further towards the periosteal surface, vascular canals form an unorganized, irregular reticulum in the middle part of compacta. These canals create primary vascular reticular bone tissue found in anterior and anteromedial sides. The remaining quadrants are composed of non–vascular bone tissue that reaches to periosteal surface.

All in all, 100 primary vascular canals of primary osteons were measured. The results are shown in *Table 1*.

DISCUSSION

According to our results the absence of secondary osteons is typical for the cortical bone microstructure of rat. This fact corresponds with *Enlow and Brown (3)* study. The authors reported that tissue resorption with subsequent disorganization seems to take place, but reconstruction into organized Haversian bone does not follow. The obtained values in *Table 1* indicate that the mean diameter of the primary vascular canal of the primary osteon (counted as arithmetic mean of minimal and maximal diameter) is 8.66±2.41 □m. We can note that rat *femur* diaphysis of Wistar breed has very short primary vascular canals of the osteons according to the classifications by *Rämsch and Zerndt* (*9*) and *Gladuhsew (5).* In the available literature we failed to find a comparable value. The remaining parameters could not be compared because of their absence in the literature. However, comparing our results with our previous investigations of rabbit (*6*) and human (*7*) cortical bone tissue microstructure brought the information that the rat *femur* diaphysis exhibits the lowest values of all the measured parameters.

Our observations seem to provide the first evidence of the qualitative and quantitative characteristics of cortical bone tissue identified in the rat *femur* diaphysis of Wistar breed. The results could be applied in archaeozoology for the identification of species from bone fragments.

CONCLUSION

We investigated qualitative and quantitative characteristics of cortical bone tissue of rat. According to our study the bone tissue is in general non–vascular. It is composed of concentric cellular lamellae; vascular canals (primary or secondary) are absent. There are some areas of primary vascular radial bone tissue in anteromedial sides and areas of primary vascular reticular bone tissue in the middle radial compacta. Haversian bone tissue is not formed. The vascular canals of primary osteons measured are very short according to the accepted classifications.

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VÝSKUM MIKROSKOPICKEJ STAVBY KORTIKÁLNEHO KOSTNÉHO TKANIVA POTKANOV

Súhrn

Detailne sme analyzovali mikroštruktúru kortikálneho kostného tkaniva 5–6 mesačných samíc potkanov z kvalitatívneho a z kvantitatívneho hľadiska. Základné rezy boli robené naprieč diafýzou v mieste najmenšej šírky stehnovej kosti. Získané kostné fragmenty boli rezané na špeciálnom mikrotóme (Leitz 1600) na konečnú hrúbku 100 □m. Zistili sme, že skúmané kortikálne kostné tkanivo je vo všeobecnosti bezcievnaté. Pozostáva z koncentrických celulárnych lamiel, cievne kanáliky (primárne alebo sekundárne) v ňom absentujú. V anteromediálnych oblastiach sa vyskytujú zóny primárneho cievnatého radiálneho kostného tkaniva a v stredovej časti kompakty zóny primárneho cievnatého retikulárneho kostného tkaniva. V rámci výskumu kvantitatívnych vlastností sme merali plochu, obvod, minimálny a maximálny priemer 100 cievnych kanálikov primárnych osteónov. Dospeli sme k záveru, že uvedené kanáliky sú veľmi úzke na základe akceptovaných klasifikácií.

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