

The structural and functional pattern of vascular bed of the osteochondral junction in femoral heads of mature rabbits

E. KLIKA¹, S. HAVELKA²

¹Praha, ²Institute of Rheumatology, Praha

SUMMARY

Klika E., Havelka S.: **The structural and functional pattern of vascular bed of the osteochondral junction in femoral heads of mature rabbits.**

Light and electron microscopical study of osteochondral junction (OCHJ) was carried out in three mature rabbits. The mineralized extracellular matrix (MEM) of the calcified layer of articular cartilage is firmly joined with the osseous tissue of bone ends. The firm connection of both components is based on a system of arciform processus of MEM dipped into the subchondral bone. On the other hand, vascular channels penetrate from subchondral bone into the MEM. Vascular channels are encapsulated by a thin mantle of osseous tissue. The terminal vascular net originating in bone marrow and dipped into the MEM of OCHJ is formed by blood sinusoids. The perisinusoidal cleft is on average 20–30 µm wide and its loose connective tissue is in close contact with extracellular matrix. The structural arrangement of the terminal vascular bed of MEM speaks in favor of a subchondral sole supply to the hyaline joint cartilage.

Findings obtained also support the idea of participation of OCHJ in remodeling of articular bone ends in mature age. The loose perisinusoidal and perivascular connective tissue is the source of differentiation of osteogenic cells which take part in the process of endochondral ossification needed for bone remodeling. The predominant parts of the surface of lacunae excavated by vascular channels in MEM of OCHJ are lined by a thin osseous layer separating MEM from the perisinusoidal loose connective tissue. The osseous mantle is considered to be a product of bone neoformation but, on the other hand, a barrier preventing the discoordination of endochondral ossification in the course of bone end remodeling.

Key words: osteochondral junction – adult rabbit hip – fine structure – role of vascular network.

SOUHRN

Klika E., Havelka S.: **Strukturální a funkční obraz cévní sítě osteochondrálního spojení na femorální hlavici dospělých králíků.**

Světelnou a elektronovou mikroskopií byla provedena studie osteochondrálního spojení (OCHJ) na femorálních hlavících u tří dospělých králíků. Mineralizovaná extracelulární matrix (MEM) kalcifikované vrstvy kloubní chrupavky je pevně spojena s kloubním koncem kosti. Toto pevné spojení je založeno na systému obloučkovitých výběžků MEM, jež se zanořují do subchondrální kosti. Z ní vnikají do MEM cévní kanálky, jež jsou obaleny tenkým pláštěm kostní tkáně. Cévy vycházejí z kostní dřevě a jejich terminální síť v MEM je tvořena sinusoidami. Perisinusoidní štěrbinu je široká průměrně 20–30 µm a její řídké pojivo je v těsném kontaktu s MEM. Strukturální uspořádání terminální cévní sítě v MEM nasvědčuje možnosti difúze roztoků do hyalinní kloubní chrupavky subchondrální cestou.

Naše nálezy podporují také představu o participaci OCHJ v remodelaci subchondrální kosti i u dospělých jedinců. Řídké perisinusoidní a perivaskulární pojivo je zdrojem diferenciaci osteogenních buněk, které se podílejí na procesu endochondrální osifikace, nezbytné pro kostní remodelaci. Převažný podíl povrchu lakun vyhloubených cévními kanálky v MEM je lemován tenkou vrstvičkou kosti, jež odděluje MEM od řídkého perisinusoidního pojiva. Tenký kostní plášť je produktem kostní neoformace, současně však jako bariéra brání diskoordinaci endochondrální osifikace během remodelace kloubního konce kosti.

Klíčová slova: osteochondrální spojení – kyčel dospělého králíka – mikrostruktura – úloha cévní sítě.

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Address of authors: Prof. MUDr. Eduard Klika, Praha, CzR, Prof. MUDr. Stanislav Havelka, Institute of Rheumatology, Na Slupi 4, 128 50 Praha, CzR

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Introduction

The osteochondral junction (OCHJ) represents e.g. in diarthroses of mature mammals including man a suitable model for morphophysiological analysis of dynamics of tissue interactions. The OCHJ contains a narrow zone of mineralized hyaline cartilage firmly joined with subchondral bone. The mineralized extracellular matrix of cartilage (MEM) of OCHJ is bordered from nonmineralized joint cartilage by one or more hematoxyphilic lines called tidemarks. Conventional histological sections, e.g. decalcified OCHJ embedded in paraffin, yield modest information concerning the fine structure of OCHJ.

The present study of femoral heads in rabbits was carried out on semithin and ultrathin durcupan sections. It was provoked by observations of Havelka and Neužil [6] who studied the samples of joint cartilage from large extremities and intervertebral discs with adjacent bone tissue from skeletally mature animals (in rabbit, dog, monkey and cow) and from humans without osteoarthrological pathology. Undemineralized material was embedded in methylmetacrylate and cut on Buehler low speed saw in sections

150–200 µm thick. Blood vessels form anastomosing network lodged in the bone marrow spaces and penetrating into the calcified layer of cartilage, but never passing the tidemark. Sequential focusing affords a three dimensional view of structures of OCHJ in thick sections.

The application of semithin sections and TEM analysis of ultrathin sections are an attempt to contribute to histotopography of OCHJ. The semithin durcupan sections are 200–400 times thinner than the methylmetacrylate slices [2]. They yield a detailed information on structure of OCHJ.

In principle, three functional roles are attributed to OCHJ:

- 1) participation in firm connexion of joint cartilage and subchondral bone,
- 2) participation of terminal vascular bed of bone marrow in metabolic exchange of joint cartilage,
- 3) remodeling of subchondral bone.

The firm connexion of cartilage and bone in OCHJ is generally accepted, the structural explanation is nevertheless lacking [1].

The role of subchondral network of terminal vascular bed in me-

tabolic exchange of joint cartilage is a matter of dispute. A group of authors stress that the vessels of subchondral bony plate play an insignificant role in nutrition of normal cartilage [7,8,12], Havelka et al. [4,5] proved by an in vivo test on exarticulated elbow joints of adult rabbit and dog that intravenously administered tetracycline can penetrate through the calcified joint cartilage via the subchondral bone already in five hours attaining the tidemark. Thus the vascular network in the joint calcified cartilage could actually support the supply of the corresponding joint cartilage.

In opinion of most authors prevails the idea according to which OCHJ participates mainly in remodeling of subchondral bone. Lemperg [11] studying subchondral plate remodeling in rabbit by microradiography and tetracycline labeling, demonstrated that remodeling, which occurs at a very rapid rate in immature rabbits, continues at a slower rate after the termination of longitudinal growth. Lane and Bullough [10] reviewed the concept of continuous growth of the articular ends of bone, starting their historical review with remarks on Ogston [13] followed by comments and citations of a series of authors. They analyzed in a longitudinal study vascularity and remodeling of subchondral bone and calcified cartilage in adult human femoral and humeral heads based on endo-

chondral ossification. They demonstrated histologically various stages of remodeling activity starting with the resorptive phase of calcified, cartilage followed by an active bone deposition. More remodeling was noted in the loaded areas at all ages. Lane and Bullough [10] consider the mineralized cartilage as the source of the growth and remodeling of the underlying bone by means of endochondral ossification in which vascular invasion from the adjacent subchondral bone produces ossification of the calcified cartilage. The thickness of the calcified zone decreases with age but the number of tidemarks increases particularly over the age 60. They suggest that the process of the remodeling of the bone is accelerated with increasing age. The SEM and TEM as well as microanalytical study of OCHJ was carried out by Hough et al. [9] in joints of humans and in joints of six other mammalian species. The long recognized absence of birefringence in the human OCHJ is attributed to the loose texture and random arrangement of its intermingling unraveled collagen fibrils which bridge the OCHJ. The tidemark, the hematoxyphilic edge of the calcified cartilage opposite the OCHJ, contained an electrondense granular interfibrillar material extractable with testicular hyaluronidase or trypsin. Clark [1] summarized the problems from several viewpoints. Most vascular

Figs. 1–6 are semithin sections (1 μ m) demonstrating the articular osteochondral junction of proximal femoral segment in rabbit.

Fig. 1

Survey picture of osteochondral junction (OCHJ); a-boundary of cartilage and bone, b-osseous vascular channels in OCHJ, c-cartilage, d-bone tissue. Azur stain. x 140.

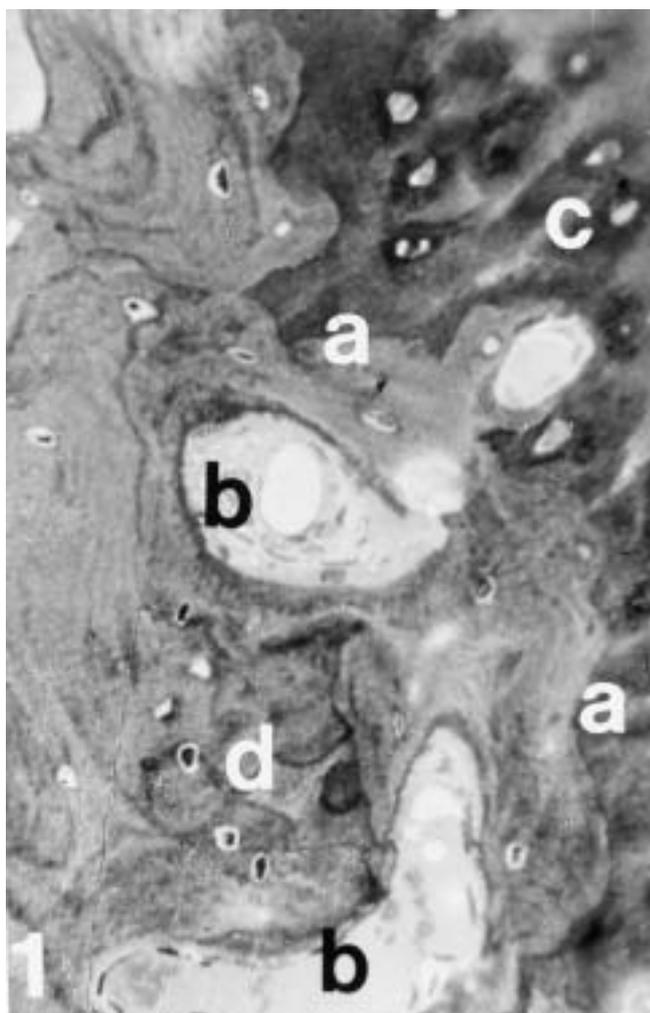
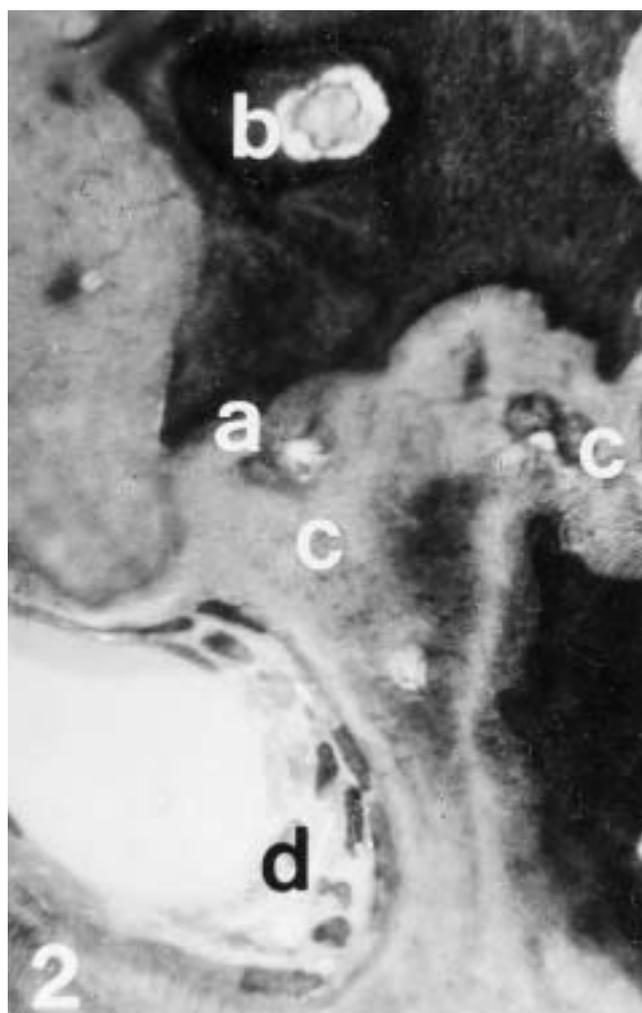


Fig. 2

Detail of OCHJ; a-mineralized extracellular matrix (MEM) of cartilage, b-chondrocyte, c-protrusion of bone tissue into the cartilage, d-osseous vascular channel with venous sinusoid and fine perivascular connective tissue. Methylene blue, basic fuchsin. x 900.



channels opening into the calcified articular cartilage were separated from the cartilage by a layer of bone. He found no support for the theory that subchondral vessels are a normal source of nutrition to articular cartilage as the vessels which actually reach the cartilage are few and small. Czech authors are among those who strongly believe in the supplementary subchondral nutritional pathway of adult articular cartilage [2,4,5]. They regard the tidemark as a special instance of the broader phenomenon of calcification / mineralization fronts. Inside the articular cartilage, tidemark changes reflect the slow remodeling of its calcified layer and its inapparent shift towards the articular surface, with tidemark reduplications increasing in number under abnormal conditions [3,5].

Materials and methods

The light and electronmicroscopical study was carried out in three mature rabbits (physes closed, body weight 3 100–3 300 g). Six femoral heads of rabbits sacrificed in nembital anesthesia were immersed at room temperature in 2,5 % glutaraldehyde solution buffered with 0,2 M sodium cacodylate at pH 7,4 and fixed for 48 hours. The specimens were rinsed then in buffer and decalcified in repeatedly changed EDTA solutions. From decalcified femoral he-

ads prismatic blocs 2 mm long and 1 mm thick were cut, the specimens were rinsed in buffer and postfixed for 12 hours at room temperature in 2 % osmium tetroxide, buffered with 0,2 m sodium cacodylate and embedded in durcupan ACM. The orientation of blocs in course of embedding process was done in such a way to enable the perpendicular sectioning through the OCHJ situated in the central part of the bloc. Semithin sections 0,5–1,0 µm thick cut on ultramicrotome LKB were stained with azure stain or by combined method with methylene blue-basic fuchsin. The modified Movat's impregnation method was successfully used in semithin sections for demonstration of connective tissues extracellular matrix (amorphous compound in particular). The application of this method enabled to demonstrate the OCHJ very distinctly. The ultrathin sections were stained with uranylacetate and lead citrate after Reynolds [14] and examined in Tesla 513 TEM.

Results

The method of semithin sections supplemented by TEM study contributed to a better understanding of organisation of terminal vascular bed in the region of OCHJ

The boundary of calcified cartilage and bone of OCHJ is not straight and forms an intricated curled line. The connexion of the

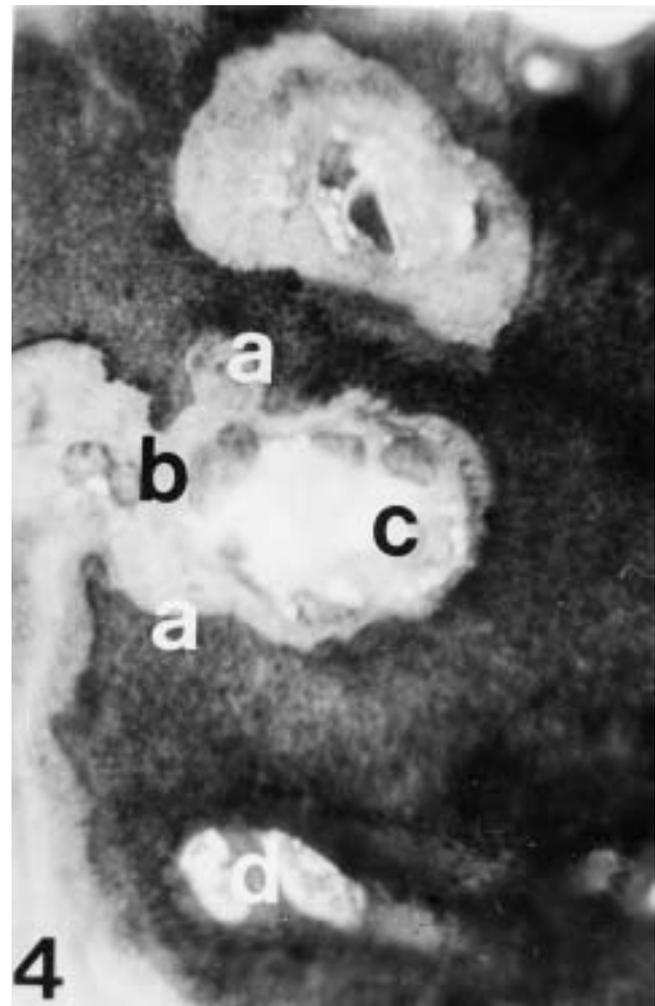
Fig. 3

OCHJ, modified Movat's silver impregnation technique; a-cartilage, b-boundary between MEM and bone, c-osseous vascular channel, d-bone tissue. x 140.



Fig. 4

Intricated course of osteochondral interface; a-dentated interface of MEM, b-bone matrix, c-top of blood sinusoid contacting the MEM of cartilage, d-chondrocyte. Methylene blue, basic fuchsin. x 900.



cartilage with the underlying bone plate has a characteristic appearance. The mineralized extracellular matrix of hyaline cartilage forms an arciform system into which penetrate vascular channels from subchondral bone, ensheathed by reticular tissue rich in connective tissue cells. The vascular channels are separated from MEM by a mantle of osseous tissue constituting a thin barrier separating MEM of hyaline cartilage from connective tissue of vascular channels (Figs. 1, 2, 3). The arciform arrangement of tissue components at the boundary of OCHJ seems to be the anchoring system which contributes substantially to a firm attachment of cartilage and bone (Figs. 1, 2, 4, 6). The staining properties of extracellular matrix of the cartilage and bone differ substantially. The sharp boundary line between them can be easily identified (Figs. 1, 2, 3, 4, 5, 6).

The vascular channels accompanied by a thin osseous mantle penetrate into the excavations in the mineralized cartilage of OCHJ. The blood sinusoids represent the terminal bed of vascular channels. The endothelium of sinusoids is provided with a thin basal lamina, in places discontinuous, and is ensheathed by a loose thin layer of reticular connective tissue in which predominate the osteoprogenitor cells (Figs. 2, 4, 7, 8). The perisinusoidal clefts are in places only 20–30 μm wide and the endothelium of sinusoids is fixed to the osseous matrix by thin retinacula of loose connective

tissue capsule of sinusoids (Figs. 7, 8). Taking into consideration the thin mantle of osseous tissue separating the hyaline cartilage from the sinusoids, it seems that this structural arrangement speaks in favour of participation of terminal vascular network in metabolic exchange of articular cartilage. This probable function support in particular the pictures demonstrating the contact of sinusoids with the cartilaginous matrix (Figs. 1, 4).

Semithin sections impregnated with Movat's silver impregnation method contribute to a better understanding of vascular bed organisation in area of OCHJ. The extracellular matrix of articular cartilage is heavily impregnated (black in colour pictures) whilst the matrix of osseous tissue appears light and is only slightly impregnated (yellow in color pictures). (Figs. 3, 5, 9). It is the extracellular matrix only lining the inner surface of osseous mantle of vascular channels which shows a distinct argyrophilia forming a dense thin layer (Fig. 5). Applying the method of Movat the penetration of vascular channels into the cartilage of OCHJ is easily visualized. On the tops of penetrating channels the osseous mantle is lacking. As it was seen on ultrathin sections, the Movat's method seems to impregnate the amorphous nonfibrillar substance of cartilaginous extracellular matrix as well as the cementing substance of lamellar bone. (Fig. 5). Movat's impregnation method, due to strong affinity to amorphous extracellular matrix, masks the

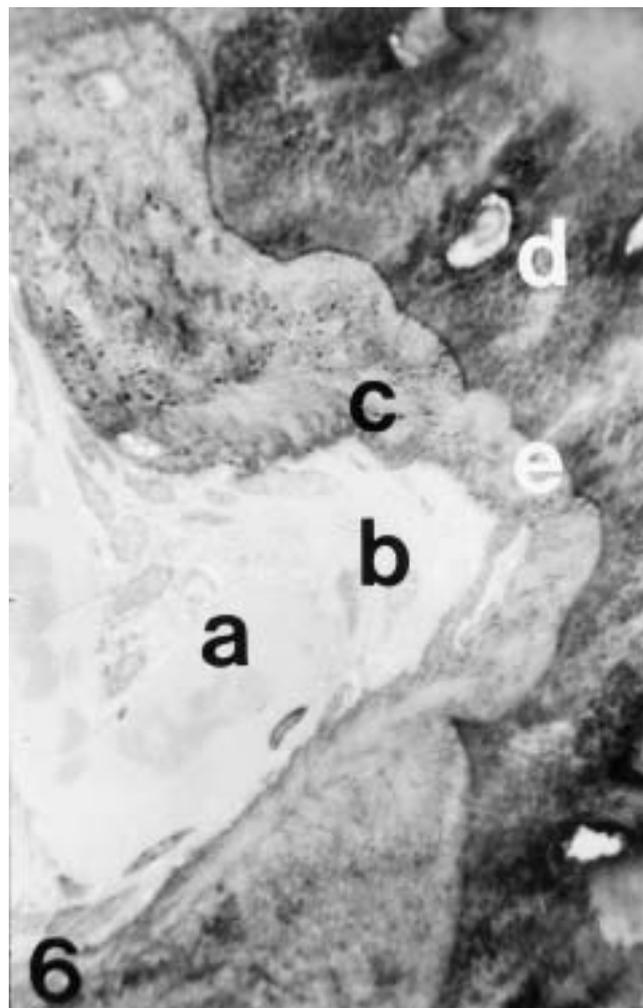
Fig. 5

OCHJ in Movat's silver impregnation technique; a-vascular channel protruding into the cartilage matrix, b-boundary of OCHJ, c-cartilage, d-the innermost layer of bone matrix is heavily impregnated, e-lamellae of bone matrix. x 350.



Fig. 6

Large vascular channel in vicinity of the calcified-noncalcified cartilage interface (a), b-perivascular connective tissue, c-layer of bone matrix, d-cartilage with chondrocytes, e-dentated line of OCHJ. Methylene blue, basic fuchsin. x 900.



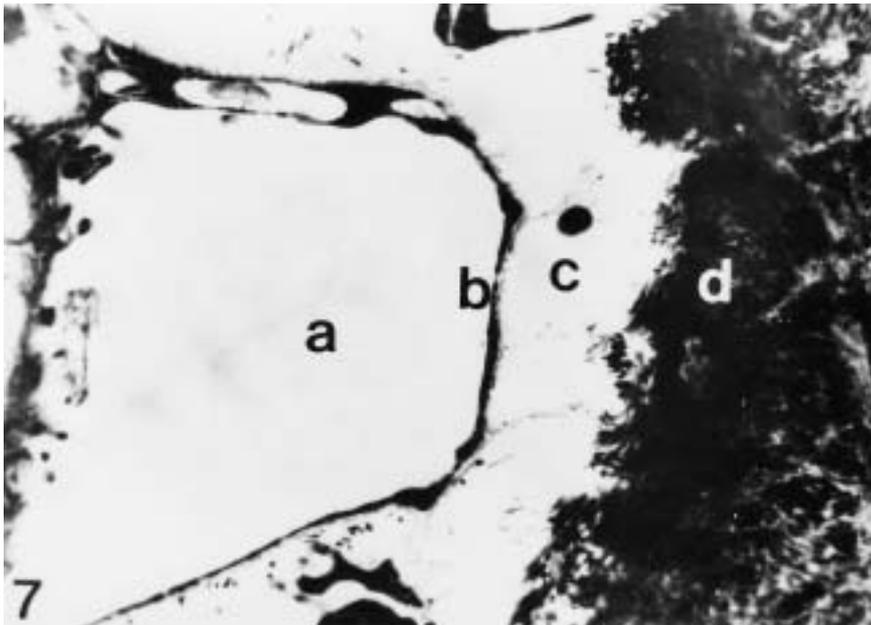


Fig. 7
Venous sinusoid of osseous vascular channel; a-lumen, b-endothelium, c-perisinusoidal space, d-dense bundles of collagenous microfibrils of bone matrix. TEM x 20 000.

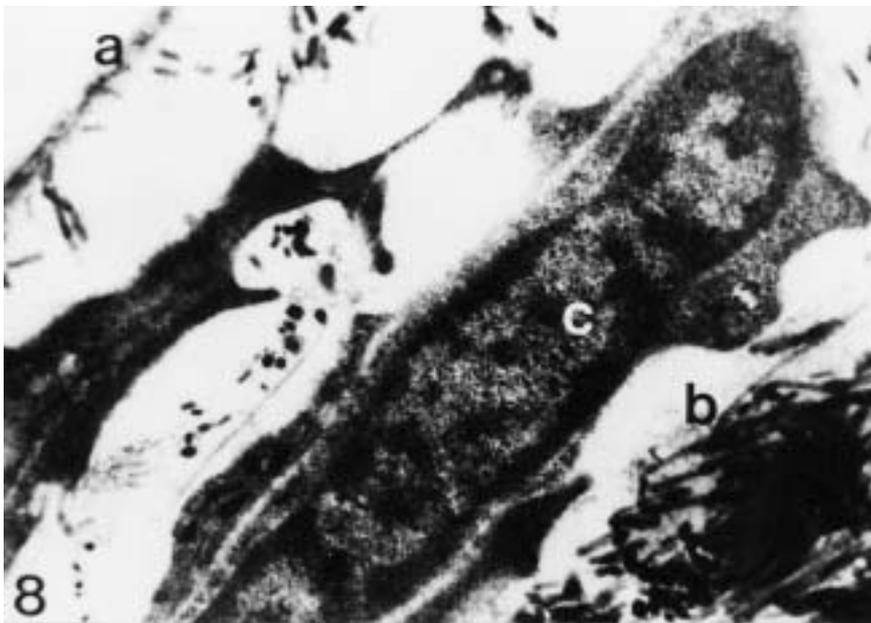


Fig. 8
The wall of a vascular channel in the region of OCHJ; a-endothelium of venous sinusoid, b-the edge of bone matrix, c-osteoprogenitor cells of perisinusoidal connective tissue. TEM x 20 000.

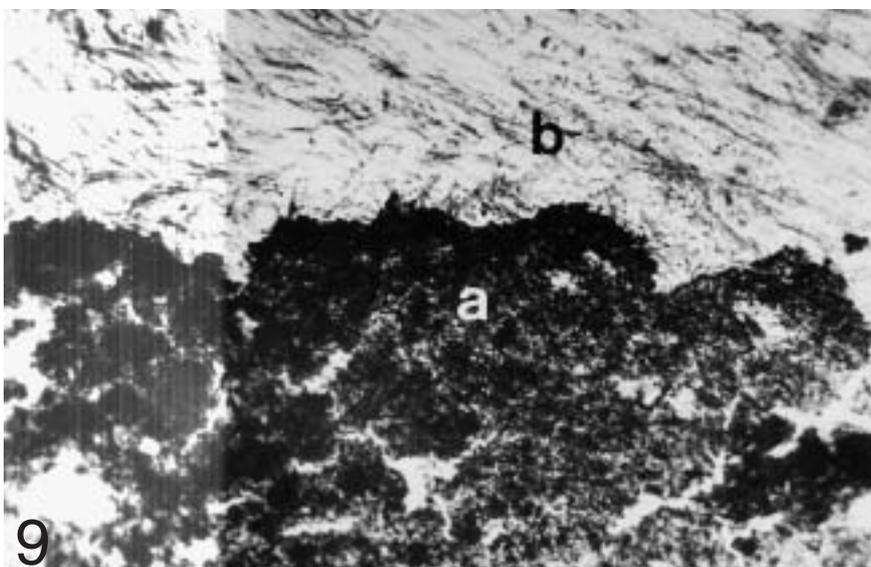


Fig. 9
OCHJ in Movat's impregnation technique; a-heavily impregnated cartilaginous matrix, b-collagenous microfibrils of bone matrix. TEM x 20 000.

collagen microfibrils of the cartilage (Fig. 9). This differs from the osseous extracellular matrix in which the compact bundles of collagen microfibrils prevail (Fig. 9).

As mentioned above, in opinion of most authors predominates the idea according to which the OCHJ participates mainly in remodeling of subchondral area of bone ends. According to our light and electronmicroscopical findings this hypothesis seems to be correct. We consider the OCHJ as „conserved“ or „quiescent“ boundary zone of endochondral ossification. The layer of mineralized hyaline cartilage is present here contacting through the thin osseous mantle the terminal vascular bed of blood sinusoids. The perisinusoidal space contains undifferentiated connective tissue cells, osteoprogenitor cells and fine reticular fibrils (Figs. 2, 6, 7, 8) These components seem to participate as osteogenetic cells in endochondral ossification in course of subchondral bone remodeling. The lacunae excavated by vascular channels in mineralized cartilage are lined by a thin osseous layer which forms a „tapestry“ separating the mineralized cartilage from the connective tissue of perisinusoidal space. We consider this osseous mantle as a product of bone neof ormation but, on the other hand, as the barrier which prevents the discoordination of endochondral ossification in course of remodeling of the subchondral bone (Figs. 2, 4, 6).

Nevertheless we observed in OCHJ places in which the sinusoids and their connective tissue sheath is found in immediate contact with MEM of cartilage. The osseous mantle is lacking here. This situation is distinctly seen especially in Fig. 4 in which the penetration of sinusoid and its connective tissue sheath into MEM is demonstrated. It is probably the place prerequisite for resorption of MEM and chondrocytes and the bone neof ormation on base of endochondral ossification.

Discussion and conclusion

The analysis of semithin sections of OCHJ enabled us to determine the structural basis of firm connexion of MEM of the cartilage and subchondral bone in the area of OCHJ. The arciform arrangement of mutually penetrating processes of MEM and subchondral bone with vascular channels represents the anchoring system of both tissues components. Their boundary itself is sharp, no massive transition of collagen fibrils between both tissue components was seen.

The questionable problem is the role of terminal vascular bed of subchondral bone in metabolic exchange of articular cartilage. Havelka et al. [4,5] put in evidence the transport of tetracycline in adult rabbit and dog which appeared five hours after intravenous application in the calcified extracellular matrix of articular cartilage. We demonstrated that the vascularisation of the subchondral bone is relatively rich. The vascularisation terminates in sinusoids dipped into the MEM of hyaline cartilage. Taking into account the close contact of sinusoids with articular cartilage in OCHJ it seems probable that this avascular bradytrophic tissue receives the nutrition and accomplishes the metabolic exchange from the terminal vascular network via diffusion and inhibition.

The findings obtained support the hypothesis which considers the OCHJ as the zone of subchondral bone remodeling. The OCHJ represents the quiescent boundary zone of endochondral ossification which is provided with all the structural „instruments“ needed for realisation of endochondral ossification, that means the participation in bone resorption and neoformation. We stress the presence of thin layer of mostly acellular bone lamellae, forming a „tapestry“ separating the MEM of cartilage from perisinusoidal connective tissue as a product of bone neoformation. On the other hand, this thin osseous mantle covering the greatest part of the wall of the lacunae of vascular channels seems to be a barrier counterbalancing the rate of bone remodeling.

The functional rules of keeping the balance in bone remodeling process are unknown. It is also important to establish the rate of proliferation of articular cartilage which undergoes resorption in course of bone neoformation.

The tidemark was not in the center of attention in our present study. As seen in *Figs. 1, 2, 4 and 6*, the tidemark is not demonstrable by methylene blue staining. It can be easily visualized in

conventional haematoxylin-eosin staining as a black-blue line. Also the Movat's impregnation method seems to be not convenient for demonstration of tidemark as the amorphous extracellular matrix of the cartilage is black, being composed of densely packed grains. In accordance with Hough, Banfield, Mottram and Sokoloff [9], the zone of tidemark is in electronmicroscope predominantly composed of electrondense granular interfibrillar material. An overview of tidemark items appeared recently [2].

In conclusion, our light and electron microscopic findings in femoral heads of adult rabbits corroborate the idea that OCHJ, with its vascular network, participates in bony end remodeling and in additional nutrition supply even to adult articular cartilage.

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