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The structure of vascular channels in the subchondral plate

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INTRODUCTION

Several theories have been offered to explain the presence of blood vessels in the subchondral layer of articular cartilage. Of particular interest are those capillaries which appear to cross the bone and penetrate the cartilage itself. While blood-borne nutrition of articular cartilage may be important during growth, various types of tracer studies have questioned this function in adults (Greenwald & Haynes, 1969; Haynes & Woods, 1975; Maroudas, Bullough, Swanson & Freeman, 1968; Ogata, Whiteside & Lesker, 1978; Ogata & Whiteside, 1979). The advancement of capillaries into the cartilage of adult joints is now thought to be a part of a remodelling process. Bullough and his associates (Bullough & Jagannath, 1983; Goodfellow & Bullough, 1967; Lane & Bullough, 1980; Lane, Villacin & Bullough, 1977; Bullough & Goodfellow, 1971) have assembled a body of data which suggests that this process is analogous to endochondral ossification and occurs normally in young and old human cartilage with an intervening period of quiescence. The invasion of new vessels into cartilage has also been described as a pathological characteristic of degenerative arthritis (Badalamente & Cherney, 1988; Harrison, Schajowicz & Trueta, 1953), rheumatoid arthritis (Greenwald & Haynes, 1969) and a response to experimental superficial lacerations (Lemperg, 1971*b*) or closed impact (Donohue, Buss, Oegema & Thompson, 1973). The differences between these physiological and pathological events are not evident.

Because bone is normally a vascular tissue, vascularity alone cannot be used to characterise the extent or type of remodelling present in a joint surface. Several investigators have observed that porosity of the subchondral bone was not equivalent to vascularity of the calcified cartilage (Lane *et al.* 1977; Green, Martin, Eanes & Sokoloff, 1970). While actual perforation of the cartilage by vessels does occur, it may be a rare event associated with distinctive morphological features. To establish exact morphological criteria for active remodelling, we studied the anatomy of capillaries which are apparently cutting into the calcified cartilage and compared them with the vascular channels confined to the adjacent subchondral bone.

MATERIALS AND METHODS

The joint surfaces studied here were harvested fresh from the tibial plateaus, patellae and femoral condyles of six mature human cadavers (ages 17–72 years), four mature dogs (physes closed) and four mature rabbits (over nine months old and physes closed). The animals were killed with sodium pentobarbitone. The medical history of the human subjects was unknown but the material studied showed no local signs of systemic disease or of any condition which would affect the joint, such as inflammation

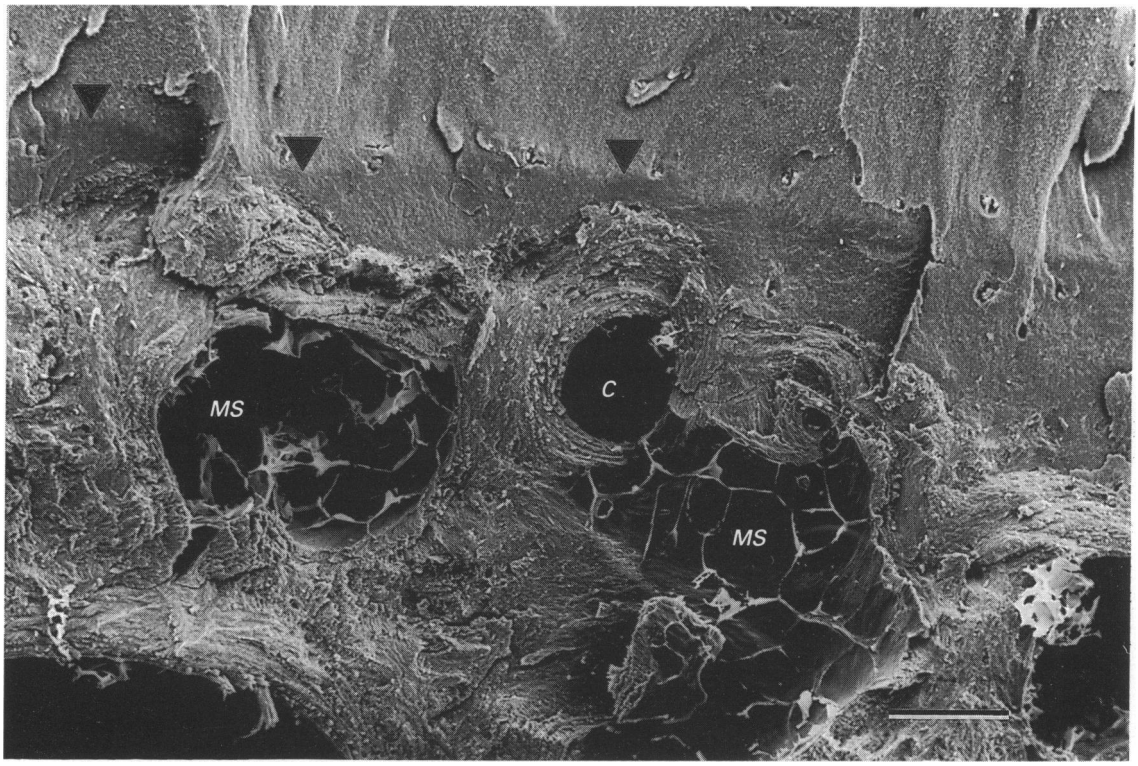

Fig. 1. Rabbit subchondral plate. The two types of large cavities in subchondral bone: irregularly shaped marrow spaces (*MS*), larger than 100 μm wide, filled with marrow fat cells and cylindrical channels (*C*), 40–100 μm diameter. Tidemark is indicated by arrowheads. Bar, 100 μm.

or disuse. The specimens were fixed by immersion into 2·0 % glutaraldehyde buffered with sodium cacodylate and adjusted with sucrose to an osmolality of 300–320 mosmol (Collins, Arbrough & Brunk, 1977), and approximately one third were decalcified in formic acid. Each human plateau was cut into halves (medial and lateral condyles). After the underlying bone of a specimen was scored with a wafering saw, it was dehydrated through graded ethanol solutions. Having reached the absolute alcohol stage, the specimen was cryofractured (Humphrey, Spurlock & Johnson, 1974, 1975) in a direction perpendicular to the articular surface while immersed in liquid nitrogen using a broad chisel placed in the saw cuts to control direction. To facilitate comparison of the periphery to the centre, the fractures were directed to create sections passing radially from the joint margin to the centre. Fractured pieces were critical point dried, mounted, gold-coated and viewed in a JEOL 35C scanning electron microscope (SEM).

RESULTS

General features

The appearance of the cartilage and bone by SEM matched previous descriptions (Clark, 1985). The border between calcified and uncalcified cartilage was distinct (Figs. 1, 2). In the rabbit, the calcified subarticular layer was especially thick, often as wide or wider than the uncalcified cartilage layer. The thickness of the human and canine subchondral plates varied with site, being greatest in the centre of the tibial plateau. The structure of the canals and vessels was similar in all species.

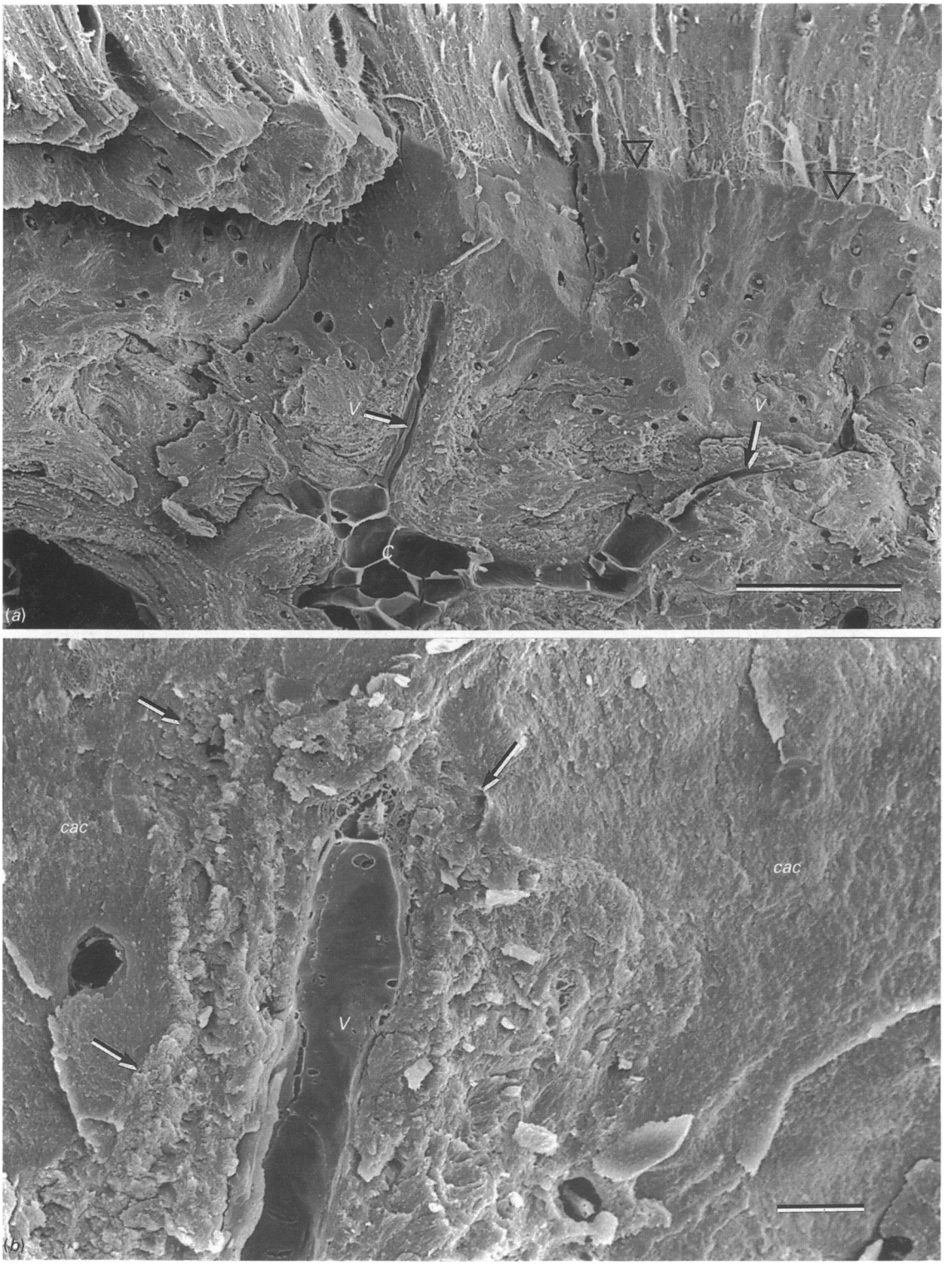

Fig. 2 (*a–b*). Canine subchondral bone. (*a*). Narrow vascular canals (*V*) branch from larger cylindrical channels (*C*). Fat cells fill the channels and narrow blood vessels run in the canals. Bar, 100 μm. (*b*) Detail of (*a*). This vascular canal contains a closed vessel (*V*), extends vertically into the calcified cartilage (*cac*), but is surrounded by a cap of bone (indicated by arrows). Bar, 10 μm.

Channels in the subchondral plate

The horizontal slab of subarticular bone and calcified cartilage was penetrated by three types of cavities. The largest were extensions of the marrow space into the subchondral bone. These spaces were more than 100 μm wide, irregular in shape and they contained fat or marrow cells (Fig. 1). Usually, these spaces were lined with flat endosteal cells. Such cavities were most common in the human material, especially near the centre of the tibial plateau, where the bone plate was thick and irregular. The second distinct type of cavity was a cylindrical canal, 30–70 μm in diameter, which passed through the bone in all directions and contained marrow cells and, occasionally, a blood vessel. These canals usually appeared as finger-like branches from larger marrow spaces. They were bordered by concentric layers of appositional lamellar bone, but did not appear to have an endosteal layer. The third type of cavity was the vascular canal, which merits a separate description.

Vascular canals

The smallest type of canal was a narrow cylinder, 10–30 μm in diameter. The canals ran in all directions and usually held one or two blood vessels (Figs. 2–4). The vessels were thin-walled and contained erythrocytes. The canals were surrounded by concentric layers of bone (Fig. 3). The bone followed the vessel, forming a sheath. In the specimens studied here, these small canals were the primary conduit for vessels in the subchondral bone.

Vertical extensions of these small vascular canals occasionally penetrated above the general level of the subchondral bone layer and into the calcified cartilage. These vertical canals were found in two forms: open and closed. In the open form, the canal contained a single capillary and was open at its leading end where it faced on to a dome-shaped cavity in the calcified cartilage (Fig. 4). The tip of the capillary was closed by a layer of endothelium. Extravascular cells lined the cavity in the calcified cartilage. The cartilage collagen fibrils ended abruptly where they came into contact with the cells and no calcified matrix surrounded the fibrils within a variable distance of the cavity. The cylindrical sleeve of lamellar bone which accompanied the canal ended short of the tip of the vessel.

In the closed form, the small canals did not open directly into the calcified cartilage, but were instead covered by a cap of lamellar bone which was continuous with that of the walls (Figs. 2, 5). Where these closed systems stood vertically, they formed knobs on the surface of the subchondral bone. In areas where these knobs extended to different heights, the interface between bone and cartilage was correspondingly irregular. This irregularity was least pronounced in the human specimens. The contents of the closed form of small canal varied. While a capillary was usually present, elongated fat cells were occasionally found within small canals.

The frequency of the two forms of small canals – closed and open – appeared to vary among specimens and with their location. The greatest concentration of open canals was found in one of the canine patellae. We did not observe any clear relationship between the pattern of vascularity in the subchondral plate and degenerative changes in the overlying cartilage. Where the bone under the centre of degenerative human tibial plateaus was thick, many channels of all sizes were present, but in numbers proportional to the volume of bone. In peripheral areas of the plateau, where the bone was thin, few channels were observed, and those appeared to be open vascular canals.

Rarely, a vascular canal actually came into contact with uncalcified articular

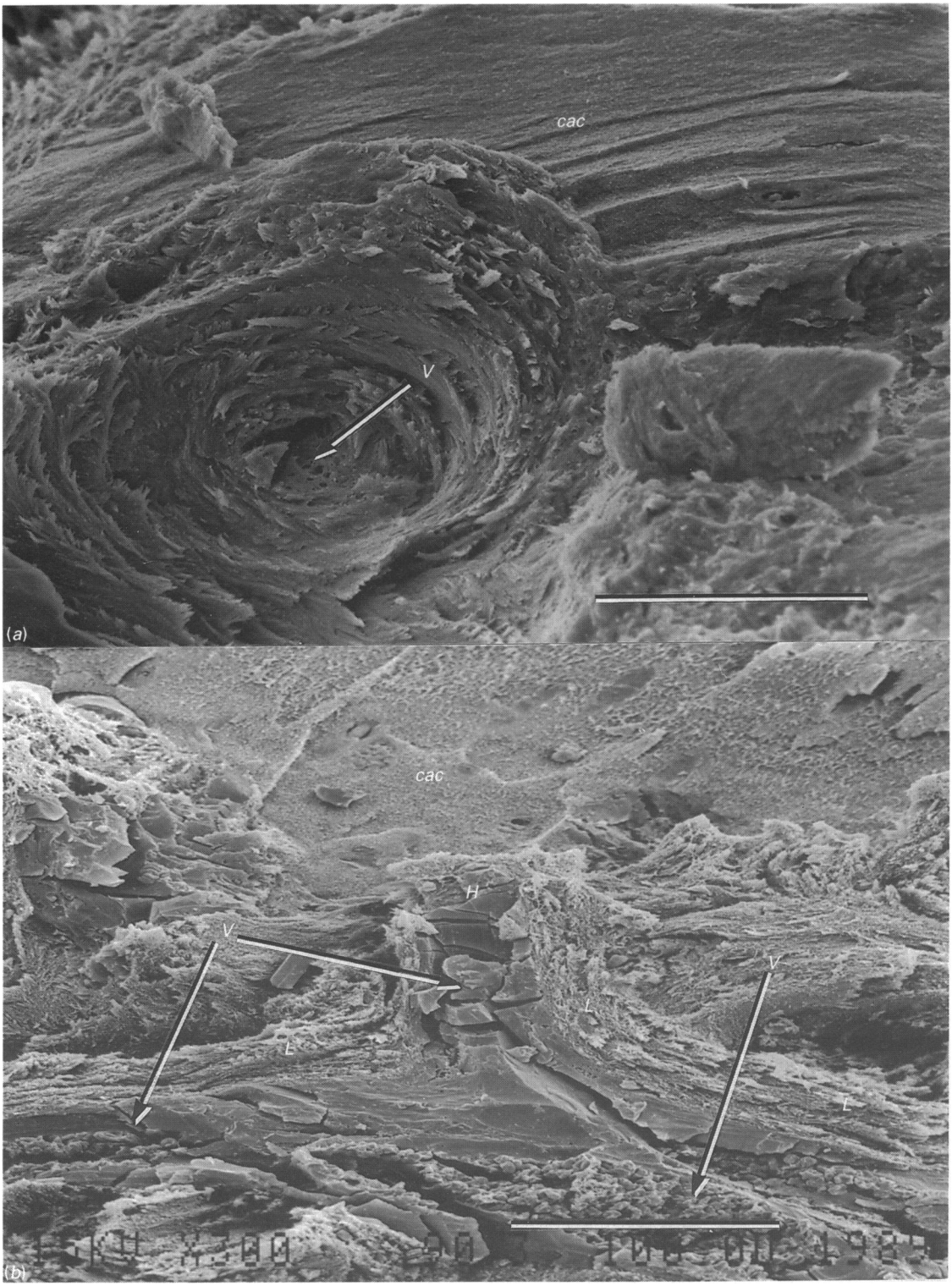

Fig. 3 (*a–b*). The structure of bone surrounding the vascular canals. (*a*) Human subchondral plate. The canal here is transected and the concentric lamellae of bone about the vessel (*V*) are exposed. The structure is typical of a Haversian system but it is in contact with calcified cartilage (*cac*). Bar, 100 μm. (*b*) Rabbit subchondral plate, decalcified specimen. Here, the vascular channel (*V*) is fractured longitudinally. A narrow canal (*H*) opens upwards towards the calcified articular cartilage (*cac*). After decalcification, the lamellae (*L*) in the bone about the vessel are more easily seen. Bar, 100 μm.

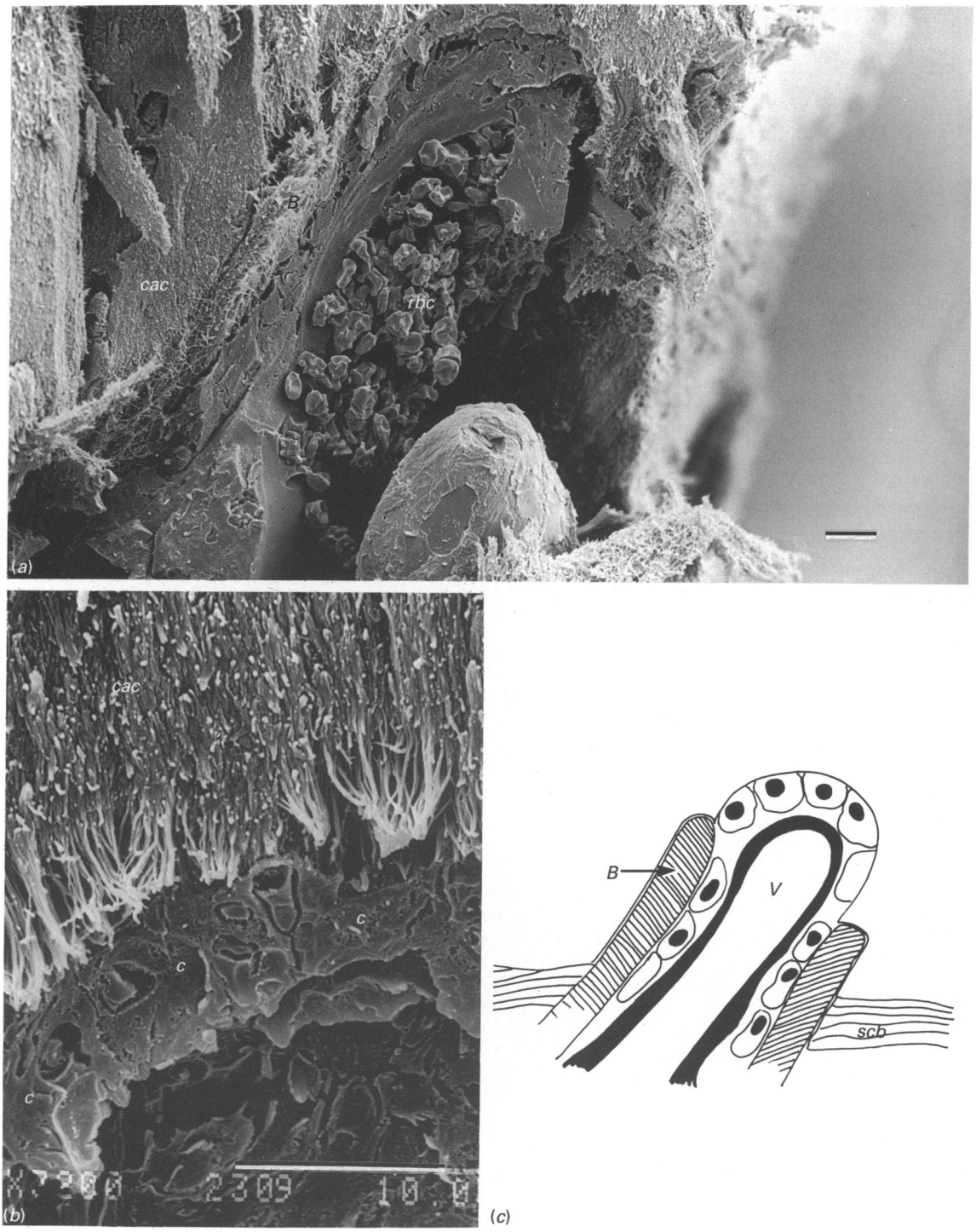

Fig. 4 (*a–c*). Open vascular canals in contact with calcified articular (*cac*), human. (*a*) The vessel, filled with erythrocytes (*rbc*), ends blindly and is encircled by a cuff of bone (*B*). Bar, 10 μm. (*b*) Detail of (*a*). The cavity at the tip of the vessel is lined with cells (*c*). The collagen fibrils in the calcified cartilage (*cac*) facing the cavity are exposed. Bar, 10 μm. (*c*) Diagram of an open canal showing the vessel (*V*), its cuff of bone (*B*) and the subchondral bone (*scb*).

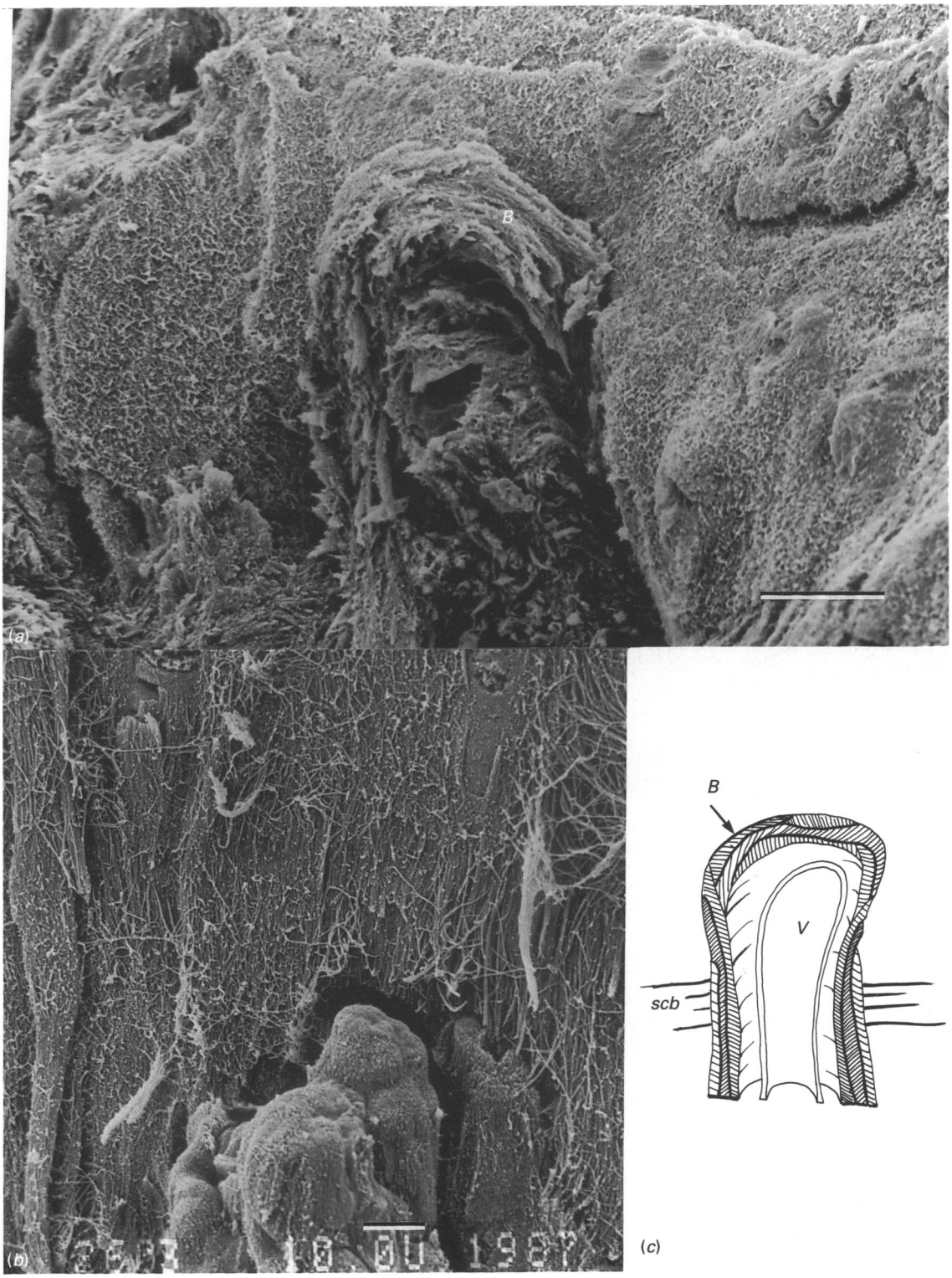

Fig. 5 (*a–c*). Closed vascular canals in the layer of calcified cartilage. (*a*) Human. This closed canal has been fractured, exposing the lamellar construction of the bony cap over the vessel. Bar, 10 μm. (*b*) Rabbit subchondral plate, decalcified. Vascular canals, capped by bone create knobs of bone extending upwards into the cartilage. After decalcification, the collagen fibres in the cartilage are visible. Bar, 10 μm. (*c*) Diagram of a closed vascular canal. The canal is capped by layers of bone (*B*) which form a process extending above the general level of the subchondral bone (*scb*) and usually contains a vessel (*V*).

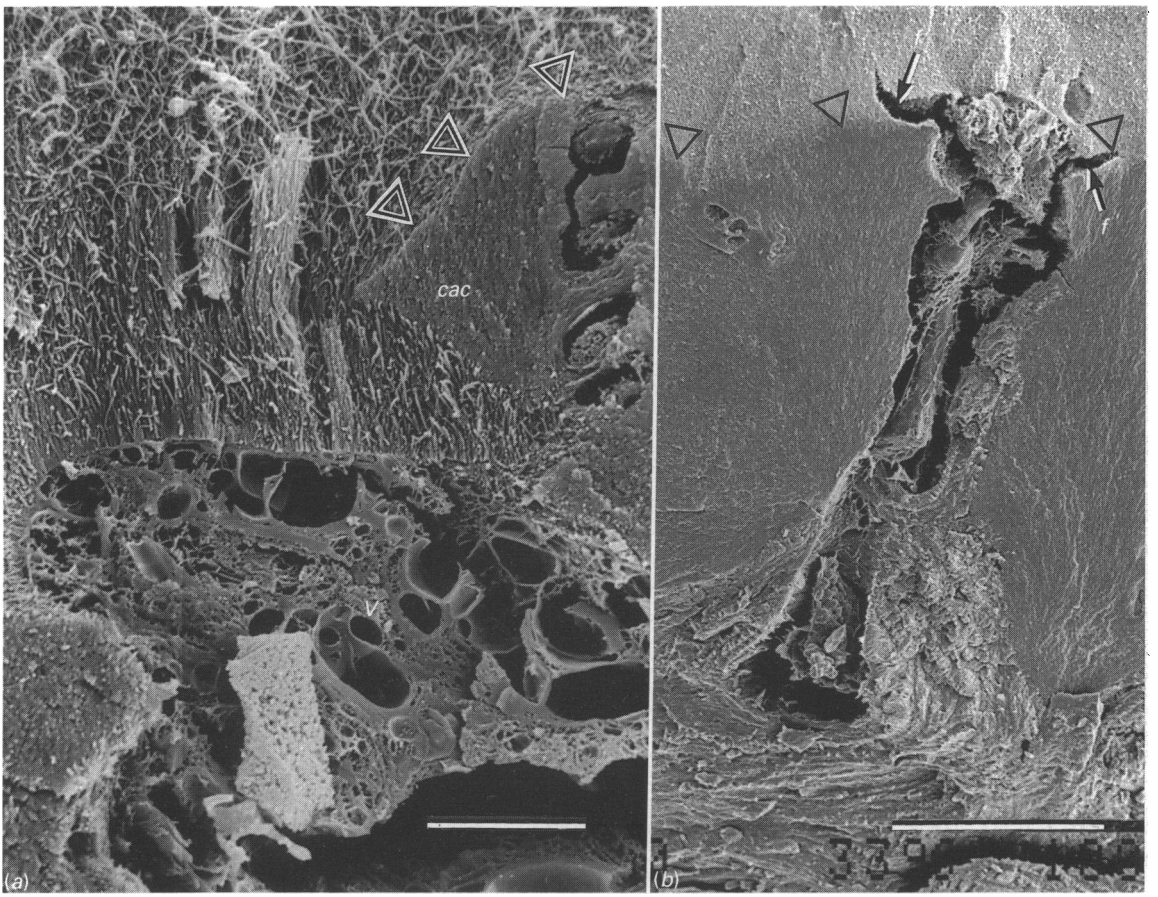

Fig. 6 (*a–b*). Examples of vascular canals which are in contact with uncalcified articular cartilage. (*a*) Here, the calcified cartilage (*cac*) at the end of the canal (*V*) was apparently decalcified, creating a dip in the tidemark (arrowheads). Bar, 10 μm. (*b*) A canal which extends across the tidemark (arrowheads). These canals were often associated with narrow fissures (*f*) between calcified and uncalcified cartilage. Bar, 100 μm.

cartilage (Fig. 6). This occurrence was usually accompanied by an apparent loss of calcification in the cartilage at the tip of the canal and a corresponding dip in the tidemark (Fig. 6*a*). No vessel encroached above the level of the tidemark. The actual structure which contacted uncalcified cartilage was the cluster of cells which preceded this capillary.

No structural feature of the adjacent overlying cartilage appeared to be correlated with the presence of penetrating vessels. Occasionally, the open vascular canals appeared to follow the planes among the collagen fibres, but vessels did not selectively invade along the cell columns. Frequently, the open canals appeared to come into contact with horizontal clefts at the tidemark or horizontal splits in the calcified cartilage (Fig. 6*b*).

DISCUSSION

Several studies have suggested that the deep surface of articular cartilage is replaced by an advancing front of bone (Bullough & Jagannath, 1983; Haynes, 1980; Lane *et al.* 1977; Lemperg 1971*a*, *b*). If, as has been hypothesised, this process is a form of endochondral ossification, then blood vessels must invade the cartilage. Histological

characterisation of the vascular canals is a way to identify and quantify this activity. Lane *et al.* (1977) have described three types of vascular canal seen by light microscopy in the subchondral cartilage: (i) those actively resorbing cartilage; (ii) those actively depositing bone and (iii) mature osteons. The SEM observations presented here support their findings but also provide the basis for modification of this concept.

We found that the perforations in subchondral bone fall into two functional groups: marrow spaces and Haversian canals. The Haversian canals were observed in mature osteons and in active osteons which were cutting a pathway through the calcified tissue and laying down bone. In the case of the latter, the SEM clearly demonstrated the capillary bud entering a cavity apparently cut from the calcified cartilage by a cluster of cells. The concentric layers of lamellar bone ensheathing the vessel are evidently formed simultaneously. Therefore we see no basis for classifying these separately as bone-forming or penetrating. Once the canal is capped with bone, it is a mature osteon, neither creating bone externally nor advancing.

Scanning electron microscopy of fractured surfaces allows an investigator to assess the bone, cartilage and vessel structure simultaneously and at high resolution. This study suggests that the degree of active vascular invasion may be overestimated by other techniques. The bony caps which denote a mature osteon are very difficult to see with the light microscope. This differentiation is also difficult on sections which have not been stained and we believe this explains the frequency of vascular penetration into cartilage described by Havelka (1986). Much of the previous SEM work has used macerated specimens (Bullough & Jagannath, 1983; Duncan *et al.* 1987; Inoue, Kodama & Fujita, 1969; Lester, Ash & Lillie, 1981). In that circumstance, the contents of a channel or pore in the plate are destroyed. It is important to know that even small (20 μm) canals can contain elements other than a vessel. Green *et al.* (1970) identified vascular channels by light microscopy which seemed to contain necrotic 'acellular plugs.' Previously unrecognised elongated lipocytes may be responsible for the appearance of subchondral 'fat emboli' on light microscopic preparations (Kawai, Tamaki & Hirohata, 1985; Fisher, Bickel & Holley, 1969). The radiographic widening of 'vascular channels' observed by Lemperg (1971*b*) following injury does not necessarily indicate vascular activity but, rather, the resorption of bone by endosteum. Such remodelling of osteons may create the 40–70 μm wide cylindrical marrow spaces observed in this study.

Most of the capillaries in the subchondral plate reside within the bone and not in the calcified articular cartilage. We found no support for the theory that subchondral vessels are a normal source of nutrition to articular cartilage. The vessels which actually reach the cartilage are few and small. The capacity of an 'end vessel' to transport nutrients is unclear, in any case. While our micrographs do not conclusively demonstrate that the capillaries which penetrate into the calcified zone are end vessels, we found no evidence that they form loops. We observed many small channels and never saw vascular loops within them. This form of blind vessel penetration is analogous to that described in the vascularisation of the cartilaginous anlage. If the vessels play a role in ossification, the process is not identical to that which occurs in the growth plate, as the vessels do not appear to follow the cell columns preferentially. The vascularisation induced by microtrauma or abnormally high stresses may be limited largely to the bone plate itself. The thick subchondral bone in the centre of degenerative human joints is highly vascular, but most of these vessels run in mature osteons and few contact calcified cartilage. It is possible that the increased vascularity associated with degenerative arthritis reflects a greater volume of normally vascular subchondral bone.

Meachim & Bentley (1978) found horizontal splits following the tidemark and

similar to those that we observed in contact with vascular canals (Fig. 6*b*). The association between horizontal and vertical clefts and active vascular canals may be an artefact of preparation, and must be examined with a technique which permits pre-existing fractures to be identified.

SUMMARY

Human, rabbit and canine articular cartilage was prepared for SEM by fixation in isosmolal glutaraldehyde and freeze-fracture following dehydration. These techniques produced clear images of the bone, marrow and vessels in the subchondral region. Generally, cavities larger than 40 μm contained elements typical of marrow. Capillaries ran through the bone in cylindrical channels 20–40 μm wide. These channels were surrounded by concentric lamellae of bone and were in all respects Haversian canals within osteons. A minority of these channels opened into the calcified articular cartilage and there were preceded by cells which appeared to be cutting into the cartilage. Most vascular channels, however, were separated from the cartilage by a layer of bone. We conclude that the vessels within subchondral bone are present primarily to supply the bone through a network of mature osteons.

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