Morphology of the Perforating Cartilage Canals in the Proximal Tibial Growth Plate of the Chick

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ABSTRACT Perforating canals arise exclusively from junctional canals just above the reserve zone and they do not branch after entering the proliferative zone. They are uniformly spaced and arranged in parallel array. The cartilage canals terminate near the beginning of the zone of hypertrophic cartilage cells. Vascular components within the perforating canals consist of a central arteriole surrounded by enlarged, interconnected capillaries which are individually in contact with the adjacent cartilage matrix. TEM shows that the capillary endothelium is extremely attenuated, possesses numerous fenestrations and lacks a continuous basement membrane. The central arteriole is enlarged through the midpart of the caal and then narrows to communicate with the capillaries near the bottom of the canal. The large capillaries ascend from their point of origin and recombine near the top of the growth plate to exit as a single venule. The vascular arrangement therefore describes a system in which the outgoing blood runs in close proximity, but counter to, the incoming blood. This vascular arrangement within the perforating cartilage canal would most likely allow the zone of maturing cartilage cells to receive the highest concentration of nutrients.

An extensive literature has accumulated on the long-recognized “cartilage canals” found in temporary or permanent cartilages of a variety of vertebrates (see reviews by Hurrell, '34; Levene, '64). The blood vessels distributed through the canals were thought to nourish adjacent cartilage and were sometimes en route to other areas. Levene ('64), in a comparative study, demonstrated that the pattern of distribution of cartilage canals was site and species specific, although others have shown that the distribution changed as the animal matured (Trueta, '57; Spira et al., '63).

Lutfi ('70b) has described the system of canals in the epiphysis of the upper end of the tibiae of growing chicks. Perforating canals arose at regular intervals from junctional canals and penetrated into the growth plate. The growth plate was defined as that region of the upper tibial cartilage with chondrocytes arranged in definite cell columns (Levene, '64; Lutfi, '70b). The descending, penetrating vessels apparently did not anastomose with ascending, metaphyseal vessels. Thus, blood delivered to the growth plate would pursue a descending afferent course and an ascending efferent course suggesting a counter-current mechanism. Although Lutfi ('70a) has shown that mesenchymal cells surrounding the blood vessels within a canal contribute to the formation of new chondrocytes, the functional significance of the perforating canals is still in doubt. Moreover, the arrangement and structure of the vascular components have not been described. It was felt that an understanding of the arrangement and structure of the vascular components of the perforating canals would elucidate their functional contributions to the chick growth plate.

MATERIALS AND METHODS

Twenty Golden Giant cockerel chicks (Jack Frost Chicks, Inc., St. Cloud, Minnesota) were killed by decapitation at four weeks of age.

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age. The right proximal tibiae were bisected longitudinally and the lateral halves fixed by immersion in 10% buffered neutral formalin for two hours and subsequently decalcified in a 20% formic acid-80% alcohol solution. After ethanol dehydration, the tissue was embedded in Tissue Embedding Solution (Randolph Products Co., Carlstadt, New Jersey). Serially sectioned at 11 microns in either the sagittal or transverse planes with a sledge microtome and routinely processed for staining with Harris’ hematoxylin and eosin.

The medial halves of the right proximal tibiae were immersed in modified Karnovsky’s fixative (Karnovsky, ’65; 1% paraformaldehyde; 1.25% gluteraldehyde; pH 7.2-4; 560 mOsM) and postfixed in 2% OsO₄, buffered in 0.072 N cacodylate buffer at pH 7.2-4 for one hour. Tissues for scanning electron microscopy were next rinsed in buffer, dehydrated in acetone, critical point dried with CO₂, sectioned again to expose a new tissue surface, mounted on specimen stubs with double-stick tape, and coated with carbon and palladium-gold. Specimens were observed in a Cambridge Stereoscan S4 scanning electron microscope using 20 kV. Similar specimens were prepared routinely for viewing in a Phillips EM-200 transmission electron microscope. Serial thick sections (approximately 1 micron) were cut from the blocks prepared for TEM and stained with toluidine-blue (1% toluidine blue; 1% sodium borate).

**Observations**

At four weeks of age the proximal tibial chondroepiphysis and growth plate of the chick are richly vascularized by way of the cartilage canals, especially along the upper margin of the reserve zone of the growth plate (fig. 1). Cartilage canals in this area are termed “junctional canals” (Lutfi, ’70b). A typical cross section of a junctional canal contains a single arteriole, one or more venules and an occasional capillary (fig. 2). Serial sections show that the small venules become confluent with the larger venule. The arteriole is small in comparison to the venules although its wall is thicker. The canal is packed with connective tissue which adjoins a narrow zone of unstained cartilage matrix.

Figure 3 illustrates a perforating canal arising from a junctional canal. The arteriole within the junctional canal can be seen to branch and enter a perforating canal in the reserve zone. The perforating canal characteristically divides into two branches that run obliquely for a distance before turning sharply downwards into the proliferative zone of the growth plate. Figure 4 illustrates the same features in an unstained whole mount. The perforating canals widen after entering the growth plate and then narrow again to terminate near the beginning of the zone of hypertrophic cartilage cells. The remnants of the canal appear in whole mounts as a thin, dark line extending from the canal through the hypertrophic zone of cartilage and in stained sections as a dense, fibrous-appearing material that frequently joins advancing marrow sprouts (figs. 5, 6).

As the canal enters the reserve zone, the venule (36 μm in diameter) occupies a large volume of the canal when seen in cross section (fig. 7). A portion of the venule wall is always in contact with the cartilage matrix. A thin endothelium and an occasional pericyte form the venule wall. The endothelial nuclei are flattened and the cytoplasm is extremely attenuated. The pericytes are found immediately subjacent to the endothelium and sometimes are interposed between the endothelium and the cartilage canal wall. The single accompanying arteriole is small (8 μm in diameter) compared to the venule. It is eccentrically placed within the perforating canal throughout the reserve zone and is usually separated from the venule by a small amount of connective tissue. The wall of the arteriole is composed of endothelium with nuclei that protrude into the lumen, and a layer of smooth muscle and connective tissue (fig. 7). A highly cellular connective tissue packs the rest of the cartilage canal at this level. Some of the connective tissue cells have long cytoplasmic processes. In toluidine-blue-stained sections, the wall of the canal is unstained. A ring of darkly stained matrix of varying width encircles the wall. Immediately beyond the ring of darkly-stained matrix, the first chondrocytes are found.

Shortly after entering the proliferative zone the single, large venule is replaced by two large capillaries, one of which is usually larger than the other. These capillaries (20-65 μm in diameter) are comprised of a layer of endothelial cells partially surrounded by pericytes. As the canal descends within the growth plate, the arteriole assumes a more central position within the canal. Branches of the capillaries...
anastomose and form a plexus of vessels around the central arteriole. Scanning electron micrographs representative of this area show that the capillaries are arranged around the periphery of the cartilage canal while the arteriole is placed in the center of the network (fig. 8). A portion of the wall of each capillary maintains contact with the canal wall (fig. 9).

As the canal penetrates deeper into the growth plate, the central arteriole increases in diameter (up to 36 μm) and becomes the largest vessel seen in cross section (fig. 10). The endothelial cell nuclei are now more elongated but not as attenuated as the endothelium of adjacent capillaries. At the level where the arteriole is enlarged, five to seven capillaries are characteristically found near the periphery of the canal, the largest of which is found in serial section to be directly continuous with the largest capillary in higher sections. Figure 11 is an electron micrograph representative of the area shown boxed in figure 10 and illustrates the attenuated nature of the capillary endothelium. The cell thickens to accommodate the mitochondria but thins abruptly to show a fenestration apparently covered by a thin diaphragm. A pericyte is interposed between a portion of the capillary endothelium and cartilage matrix. A continuous basement membrane is lacking.

As the canal descends deeper into the growth plate, the expanding lacuna cause the cartilage canal to lose its circular form as illustrated by scanning electron microscopy in figure 12. Adjacent cartilage cells are arranged in a spokelike fashion around the canal. The central arteriole undergoes a drastic reduction in size, but maintains a central position within the canal. Figure 13 is an enlarged view of the central arteriole. The endothelial cells bulge into the lumen of the arteriole and appear to be orientated longitudinally within the vessel. Figure 14 represents a cross section of the arteriole at the same level as seen by transmission electron microscopy. The thick endothelial cells bulge into the lumen. Pericytes surround the arteriole and are separated from the endothelium by a basement membrane. Adjacent endothelial cells are joined by "tight" junctions.

The central arteriole joins the capillary network near the bottom of the canal. The space below its termination is filled with connective tissue, as seen in figure 15. The continual decrease in the overall size of the cartilage canal occludes the vessels. At this point, therefore, the walls of the cartilage canal are in close apposition. In cross section, the occluded canal appears to be filled with a fibrinoid material and cellular debris. The surrounding enlarged chondrocytes appear to have a marked effect on the outline of the canal (fig. 16).

Marrow sprouts invade the hypertrophic cartilage in two ways (fig. 17). Some marrow sprouts utilize the scar of the occluded canal as a pathway while others simply penetrate the hypertrophic cartilage about half-way between the scars of occluded perforating canals.

**DISCUSSION**

The findings of this study indicate that the vascular components of the perforating canals form a complex system in which the incoming blood runs in close proximity, but counter to, the outgoing blood. A single arteriole and venule are present within the perforating canal as it branches from a junctional canal and enters the growth plate. Shortly after penetrating the proliferative zone, the arteriole becomes centrally located within the canal surrounded by five to seven large, anastomosing capillaries that have a segment of their walls in direct contact with the wall of the cartilage canal. Somewhat deeper within the plate, the central arteriole increases greatly in diameter and then narrows drastically before communicating with the capillaries just above the point where the cartilage canal is undergoing occlusion. Thus blood enters the system through a single arteriole and is emptied into a comparatively large system of capillaries that recombine to leave the growth plate as a single venule. In the systemic vascular system, the diameter of individual blood vessels decrease as a function of distance from the heart, but the total volume of the vascular system is increased. Thus, blood pressure and rate of flow are decreased as a function of distance from the heart (Guyton, '76). Most likely then, the rate of flow and blood pressure within the enlarged capillaries is considerably lower than in the arteriole.

Although the precise details of the physiological exchange that occurs between the blood and cartilage matrix are not known, it appears that the structure and arrangement of the vascular system within the perforating canals is extremely important to the continuing function of the chick growth plate. Physiological exchange is probably enhanced by the structure of the endothelium of the enlarged
capillaries which is greatly attenuated and fenestrated and possesses no continuous basement membrane. The vascular arrangement suggests that the highest concentration of nutrients would be found in the capillaries near the bottom of the canal. As the capillaries ascend within the perforating canal through the proliferative zone, the concentration of nutrients would most likely decrease by the process of diffusion through the fenestrated endothelium to the surrounding cartilage matrix. The opposite would be true for the metabolic products of the chondrocytes. The zone of maturing chondrocytes may therefore receive the highest concentration of nutrients.

The perforating canals appear to be occluded at their distal ends by the adjacent hypertrophying chondrocytes. As the chondrocytes enlarge, their lacunar walls appear to press against the wall of the cartilage canal until the canal is occluded. The chondrocytes fill the space previously occupied by the cartilage canal, accounting in part for their spikelike arrangement around the occluded canal. Concurrent occlusion of the vessels within the canal also occurs. The compact fibrinoid material seen in the occluded canal probably represents the collapsed, degenerated vascular components of the canal. The arrangement of the vessels within the distal part of individual perforating canals is never identical and the arrangement probably represents the unique accommodation of blood vessels within each canal to occlusion of that canal.

Certain morphological similarities exist between perforating and epiphyseal cartilage canals. Watermann ('66) describes a cartilage canal system within the distal femoral epiphysis of the neonatal human in which the large canals often contain a centrally placed arteriole surrounded by large capillaries and one or more venules. Other canals contain only a network of wide capillaries. The endothelial lining of many of these peripherally arranged capillaries often lies immediately adjacent to the cartilage matrix. The "thin-walled vessels" found within the humoral and femoral epiphyseal cartilage canals of fetal sheep are composed of thin endothelial cells which lack an adjacent basement membrane and are usually found directly opposed to the cartilage matrix (Stockwell, '71). Various organelles including mitochondria and "rough surfaced" endoplasmic reticulum are present within the thicker regions of the endothelial cells. Other regions of these cells contain pores, 60 nm in diameter, which are covered by a diaphragm.

In contrast to the epiphyseal cartilage canals described by various investigators (Haines, '33; Hurrell, '34; Levene, '64) the perforating cartilage canals apparently arise exclusively from the junctional canals and they do not branch after entering the proliferative zone of the growth plate. Cross sections of the epiphyseal plate show that canals are uniformly spaced (0.249 mm) and longitudinal sections show that they are arranged in a parallel fashion. The canals in the chondroepiphysis of the pup (Wilsman and Van Sickle, '72) exhibit only a segmental distribution with an average distance of 1.42 mm between canals. The perforating canals do not appear to be directly involved in osteogenesis as do the epiphysial cartilage canals (Mashuga, '61; Wilsman and Van Sickle, '70) although indirectly their nutrient function should have a role in the calcification of the growth plate.

The arrangement and pattern of the perforating canals is also important. Marrow sprouts advance into the growth plate along the scars of the perforating canals or about midway between the scars of adjacent perforating canals. This mode of advancement probably accounts for the uniformly spaced narrow sprouts seen by Wolbach and Hedges ('52). Thus, the initial lattice-work of medullary bone in the metaphysis is determined indirectly by the arrangement of the perforating canals.

LITERATURE CITED


Haines, R. W. 1933 Cartilage canals. J. Anat., 68: 45-64.


Abbreviations

A, Arteriole
C, Capillary
CA, Central arteriole
CC, Cartilage canal
CD, Cellular debris
CM, Cartilage matrix
CT, Connective tissue
CW, Canal wall
E, Chondroepiphysis
EC, Endothelial cell
ECA, Enlarged central arteriole
F, Fenestration
FM, Fibrinoid material
GP, Growth plate
HC, Hypertrophying chondrocyte
HZ, Hypertrophic zone of the growth plate
JC, Junctional canal
M, Metaphysis
MI, Mitochondrion
MS, Marrow sprout
OPC, Occluded perforating canal
P, Pericyte
PC, Perforating canal
PZ, Proliferative zone of the growth plate
RZ, Reserve zone of the growth plate
SOC, Secondary ossification center
V, Venule

PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section of proximal chick tibia. Cartilage canals (CC) supply the chondroepiphysis (E), secondary ossification center (SOC) and growth plate (GP) of the proximal chick tibia. Junctional cartilage canals (JC) lie along the upper margin of the reserve zone (RZ) and give rise to perforating canals (PC) which penetrate the proliferative zone (PZ) and are occluded in the hypertrophic zone (HZ). Scars of occluded canals appear to guide marrow sprouts (MS) from the metaphysis (M) into the growth plate. Line drawing. × 9.

2 Cross section of a junctional canal. The canal (JC) is bounded superiorly by the chondroepiphysis (E) and inferiorly by the reserve zone (RZ) of the growth plate. Vascular contents of the canal include a capillary (C), a single arteriole (A) and two venules. Serial sections reveal that the smaller venule (V1) joins the larger venule (V2). Hematoxylin and eosin. × 800.

3 Junctional canal giving rise to a perforating canal. The perforating canal (PC) passes through the reserve zone (RZ) and proliferative zone (PZ) and becomes occluded at the beginning of the hypertrophic zone (HZ). JC, Junctional canal. Hematoxylin and eosin. × 180.
PLATE 2

EXPLANATION OF FIGURES

4 Whole mount showing perforating canals. The growth plate is bounded by the chondroepiphysis (E) superiorly and by the metaphysis (M) inferiorly. The perforating canal (PC) originates in the reserve zone (RZ), passes through the proliferative zone (PZ) and becomes occluded in the hypertrophic zone (HZ) of the growth plate. Marrow sprouts (MS) invade the hypertrophic zone from the metaphysis. Unstained. × 36.

5 Whole mount showing termination of perforating canals. The perforating canals pass through the proliferative zone (PZ) and become occluded (OPC) in the hypertrophic zone (HZ). MS, marrow sprout. Unstained. × 32.

6 Marrow sprout invading hypertrophic zone. The occluded perforating canal (OPC) serves as a pathway for invasion of the hypertrophic zone (HZ) by the marrow sprout (MS). PC, perforating canal. Hematoxylin and eosin. × 650.

7 Cross section of perforating canal in reserve zone. The arteriole (A) and venule (V) are eccentrically placed in the canal at this level. Dense connective tissue (CT) fills the rest of the canal. The canal wall is composed of an unstained material (arrows). Toluidine-blue. × 1,730.
PLATE 3

EXPLANATION OF FIGURES

8 Scanning electron micrograph of perforating canal in upper proliferative zone. The arteriole (A) is moving to a more central position within the canal and branches of the capillaries (C) anastomose to form a plexus of vessels around the arteriole. \( \times 840 \).

9 Scanning electron micrograph of capillary in upper proliferative zone. Capillaries (C) surround the arteriole (A) and some of their endothelial cells (EC) maintain close contact with the canal wall (CW). \( \times 900 \).
PLATE 4
EXPLANATION OF FIGURES

10 Cross section of perforating canal in lower proliferative zone. Five to seven capillaries (C) surround the enlarged central arteriole (ECA) at this level. Toluidine-blue. $\times$ 1,360.

11 Transmission electron micrograph representative of the area shown boxed in figure 10. The attenuated endothelial cell (EC) thickens to accommodate the mitochondria (MI) but thins abruptly to show a fenestration (F) apparently covered by a thin diaphragm. A pericyte (P) is interposed between a portion of the capillary endothelium and cartilage matrix (CM). $\times$ 40,280.

12 Scanning electron micrograph of a perforating canal in the extreme upper limit of the hypertrophic zone. As the chondrocytes begin to hypertrophy (HC), the central arteriole (CA) and the capillaries (C) begin to decrease in size. Adjacent cartilage cells are arranged in spokelike fashion around the canal (lines). $\times$ 1,400.

13 Scanning electron micrograph of central arteriole in upper hypertrophic zone. Endothelial cells (EC) bulge into the lumen of the central arteriole (CA) and appear to be orientated longitudinally within the vessel. $\times$ 1,500.
PLATE 5

EXPLANATION OF FIGURES

14 Transmission electron micrograph of central arteriole in upper hypertrophic zone. Thick endothelial cells (EC) bulge into the lumen of the central arteriole (CA). Pericytes (P) surround the arteriole. Adjacent endothelial cells are joined by "tight" junctions (arrows). × 1,800.

15 Cross section of a perforating canal in upper hypertrophic zone. Connective tissue (CT) fills the space below the termination of the central arteriole. Peripheral capillaries (C) are still present. Toluidine blue. × 880.

16 Transmission electron micrograph of perforating canal in middle hypertrophic zone. The occluded perforating canal (OPC) is filled with a fibrinoid material (FM) and cellular debris (CD). The surrounding hypertrophic chondrocytes (HC) appear to have a marked effect on the outline of the canal. × 3,390.

17 Marrow sprouts invading lower hypertrophic zone. Marrow sprouts are invading the hypertrophic zone by two means. Some advancing marrow sprouts (MS) are using the scars of the occluded canals as a pathway for invasion. Other marrow sprouts (MS) are simply penetrating the hypertrophic zone between adjacent occluded perforating canals. Hematoxylin and eosin. × 160.