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Development and segments of cartilage canals in the chick embryo. A light microscope study

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Summary

Serial histological sections of distal femoral and proximal tibial epiphyses from 24 chick embryos with ages between the 8th and the 21st day of incubation were studied by light microscopic examination. Cartilage canals first appeared on the 9th and 10th day in the femur and tibia, respectively, as invaginations of the perichondrium. A completely developed canal is composed of three different segments: first, a proximal and funnel-shaped segment that retains a perichondral lining; second, a medium and tubular segment with no clear wall boundary; and third, a distal segment which shows a discontinuous wall structure composed of a fibrillar layer and a cellular layer; this segment features a globular end characterized by the absence of wall structure but rather, a close apposition of the canal's cellular elements with the neighboring cartilage matrix. The relationships of the canals and the secondary centres of ossification are commented.

Key Words: Cartilage canals - Chick embryo - Femoral epiphysis - Tibial epiphysis.

Introduction

The development of cartilage canals, and their relationship with the secondary centres of ossification have been studied in chicks (Lutfi, 1970; Hunt el al., 1979), dogs (Wilsman and Van Sickle, 1970), sheep (Stockwell, 1971), rats and rabbits (Kugler et al., 1979). Later research was directed towards the structure of the cartilage canals and the relationship between the elements within the canal and adjacent cartilaginous tissue. Several investigations in humans (Rodríguez et al., 1985; Chappard et al., 1986; Chandraraj and Briggs, 1988), mice (Cole and Wezeman, 1985; Cole and Cole, 1989), rats (Cole and Cole, 1989; Delgado-Baeza et al., 1991), marsupials (Thorp, 1990) and chicks (Abd El-Aziz, 1998) have reported the presence of different types of cells in the connective tissue surrounding the canal vessels and have also described the chondroblastic and chondrolytic activities occurring in the boundary zone between the canals and the adjacent cartilage matrix. The present work offers a light microscopic study of the three segments of the cartilage canal wall in developing epiphyses of chick long bones.

Materials and Methods

Both sides distal femoral and proximal tibial extremities were obtained from twenty-four 8-21-dayold chick embryos (White-Leghorn). The lower limbs were amputated and dissected before fixing in formalin solution (10%). After fixation, the limbs from specimens older than 12 days were decalcified by means of formic acid, dehydrated through graded ethanol series and embedded in paraffin. Serial 10 (μm thick sections were cut and stained after rehydration by Ehrlich's haematoxylin and eosin. Under ether anaesthesia and prior to fixation, two embryos of 16 and 20 days of age were injected with indian ink inside the left ventricle through its anterior wall after cutting the right atrium. Sections were studied by light microscopic examination.

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Results

Cartilage canals developed as invaginations from the perichondrium that were accompanied by the inner proliferative zone of that membrane. The invaginations, which appeared on the 9th day in the distal epiphysis of the femur (DEF), grew from the perichondrium of the intercondylar fossa (Fig. 1). In the 11-day old embryos, canals were seen in both the lateral and medial faces of the DEF. In the proximal epiphysis of the tibia (PET), invaginations from the intercondylar and peripheral regions of the perichondrium appeared in the 10-day old embryos. Capillaries were observed in both the DEF and PET cartilage canals of 10-day old embryos. Arteries and veins were seen in 11-day old embryos. No structural changes occurred after the 12th day except for an increase in the number of canal branches. Neither were any new canals developed. On the 13th day of development, perforating canals arose from the cartilage canal situated closest to the proliferative zone of the cartilage. The perforating cartilage canals penetrated the growth plate, crossed it, and continued deeper into the diaphysial cartilage in a parallel array to the longitudinal axis of the bone. The diameter of the perforating canals was twice that of the epiphysial canals and communicated with the blood vessels which growing out from the diaphysis (Fig. 2).

Cartilage canals were observed to consist of three differently structured segments. At its origin on the cartilage matrix surface, the first segment was characterized by a funnel-shaped canal, where a similar perichondral tissue was seen (Fig. 3). The second segment was tunnel-like; it was usually rough and branched, and penetrated deeper into the cartilage matrix; the third segment was the distal one, wider, blind-ended and drumstick-shaped (Figs. 5 and 6). In most cases, it was not possible to distinguish the boundary between the elements inside the canal and adjacent epiphysial cartilage. In specimens older than 14 days, the parietal structure, composed of a compact layer with fibrillar aspect between the connective tissue within the canal lumen and the cartilage cells of the matrix, was only present in the distal segment of cartilage canals. In this area, the spindle-shaped cartilage cells were arranged in parallel to the canal axis, in contrast to cells located more peripherally around the canal. This arrangement was only seen in regions where the fibrillar-like layer was clearly defined (Fig. 4). This wall structure became less obvious as the canal continued deeper into the epiphysis. There was no clear limit between the drumstick terminal ends and the surrounding cartilage tissue. Instead, the cartilage was invaded by the tissue inside the canal, especially in the distal zone (Fig. 5). The cartilage matrix had no characteristic structure. In other same-aged canals, this surrounding matrix showed an amorphous appearance with a paucity of cells (Fig. 6).

A typical content was seen in a developed canal, composed of a core of reticular tissue in which two vessels, arteriole and venule, were found (Fig. 7). A loose connective tissue containing numerous capillaries surrounded these vessels. The specimens injected with indian ink showed the perichondral origin of these vessels (Fig. 8). The bulked distal end was occupied by a glomerulus-like capillary net (Figs. 5 and 6).

Discussion

Regarding the date of origin of the canals, our observations are in agreement with the histological studies of Lutfi (1970), but they are in disagreement with those of Doménech-Ratto et al. (1992), obtained using indian ink injection, who observed them one day later. We believe that the lack of permeated vessels in the initial development of the canal does not allow observation of the canals by this method. Neither are our data in agreement with the observation from Abd El-Aziz (1998), who described the origin of the canals in the PET in 13-day old embryos.

With respect to the structure and development of the cartilage canals, our observations suggest that the morphological characteristics of the canals change in relation to two parameters. On the one hand, the time since the appearance of the canal and, on the other, the distance from the canal origin. Thus, during the earlier stages we already noted its composition based on elements with the aspect of perichondral tissue, which form the initial bud of a canal. After penetration of blood vessels from the perichondrium into this bud, the canal grows deeper into the cartilage matrix, and the perichondral tissue persists, giving a funnel-shaped entrance to the canal. These observations are in disagreement with findings of Delgado-Baeza et al. (1991), who suggested that the canal is not itself a continuation of the perichondrium, which is not true for the proximal perichondral segment.

During canal progression into the cartilage, there is an initial phase in which no clear boundary with the surrounding cartilage matrix can be found. In the following phase, the canal is endowed with cellular wall whose elements are disposed longitudinally to the canal axis and are separated from the canal lumen by a fibrillar-like layer. This disposition has already been described by Stockwell (1971) in the deep canals of proximal femoral epiphyses of sheep fetuses. Otherwise, this author noted that an absence of both cellular and fibrillar layers in the superficial

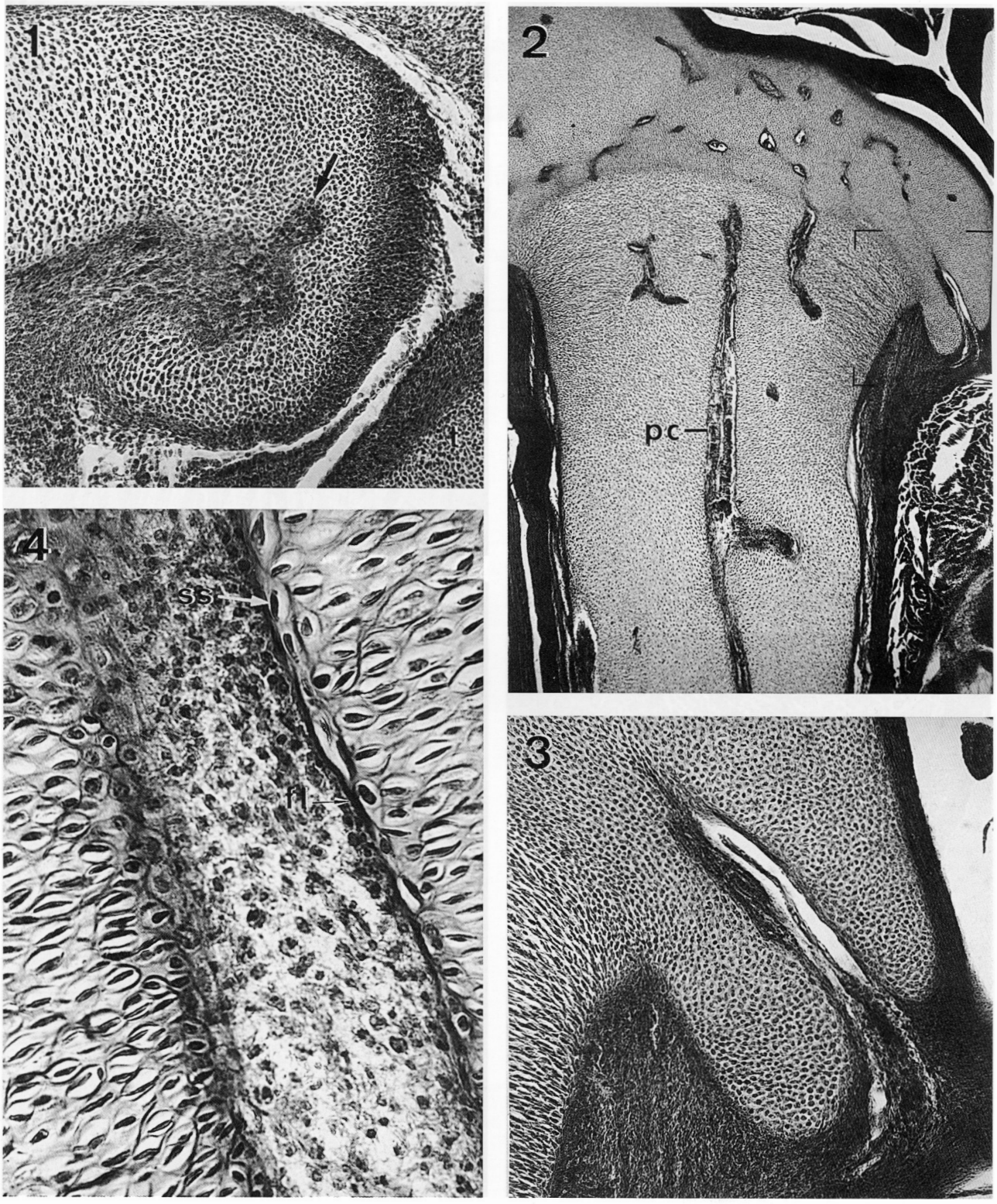
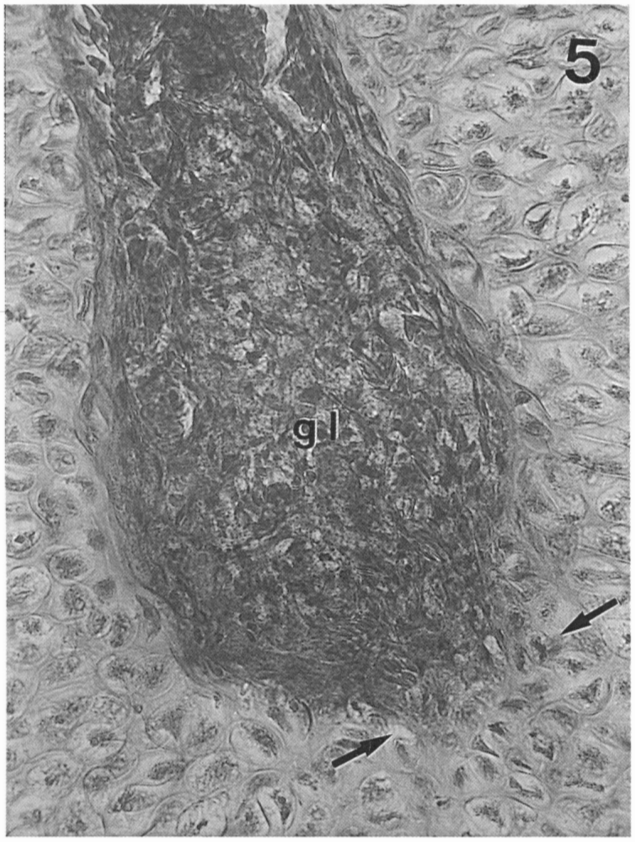
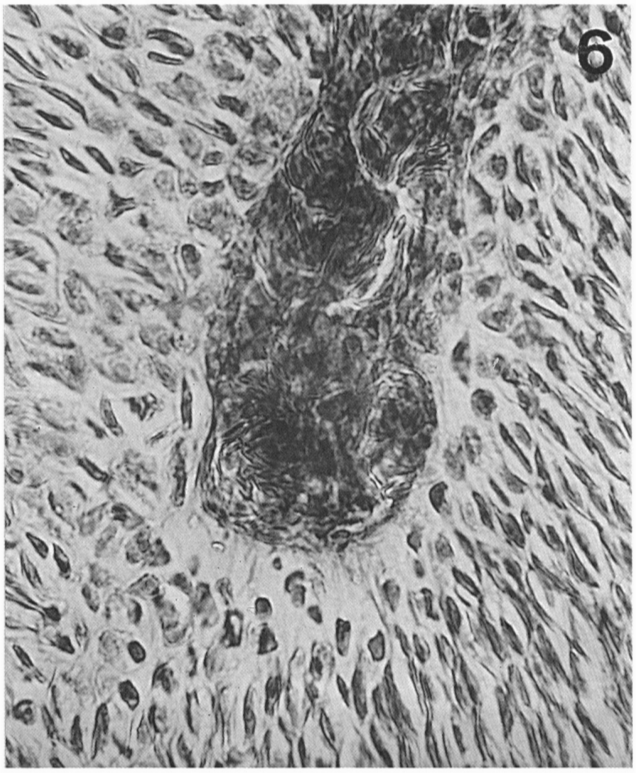
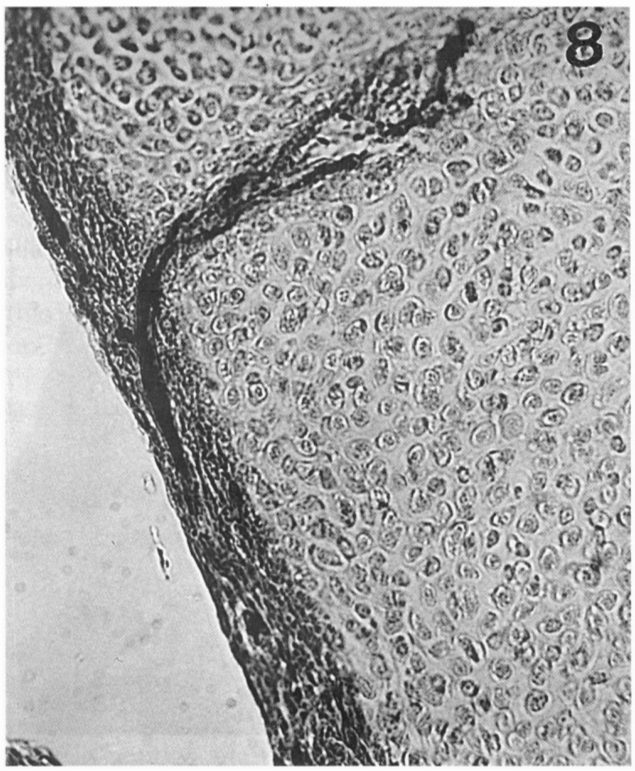


Fig. 1.– A 9-dayold DEF (x 160). Parasagittal section. Perichondral bud in the intercondylar fossa (arrow). **t,** tibia.
Fig. 2.– A 15-dayold PET (x 40). Longitudinal section. **pc,** perforating canal.
Fig. 3.– A 15-dayold PET (x 160). Detail from fig. 2.
Fig. 4.– A 21-dayold PET (x 400). Lower end of a perforating canal. **fl,** fibrillar layer; **ss,** spindle-shaped cells.







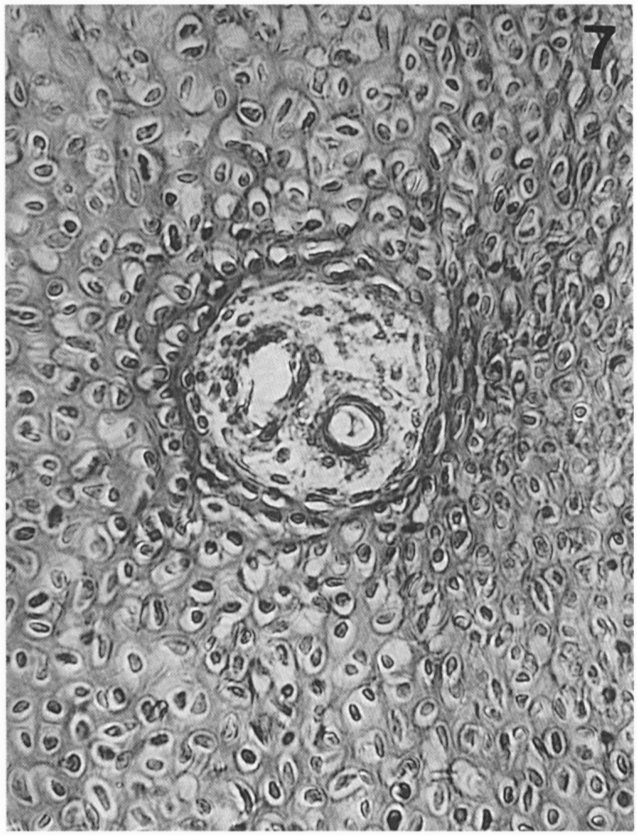


Fig. 5.– A 15-dayold PET (x 400). Drumstick-like end of a canal. Note, between arrows, the absence of a limit between the glomerulus **(gl)** and the cartilage matrix **(cm)**
Fig. 6.– A 15-dayold PET (x 400). Drumstick-like end of a canal. Note the paucity of cels in the adjacent cartilage matrix.
Fig. 7.– A 21-dayold DEF (x 400). Transversal section of a cartilage canal with its content.
Fig. 8.– A 16-dayold PET (x 400). Indian ink intravascular injection. The blood wessels arise from the perichondral wessels.

canals is most common. Lutfi (1970) does not describe any wall element as a limit to the canal lumen in chicken. We found them in canals from specimens older than 14 days, but not throughout the canal. At sites places where this structure is absent, the deeper cells of the cartilage form the limit of the canal lumen. It is possible that these cells could play a role in the appositional growth of the surrounding cartilaginous matrix from the margins of the cartilage canals (Wilsman and Van Sickle, 1970). Neither did Hunt et al. (1979) describe a fibrillar layer wall between the capillaries of the canal and the neighboring cartilaginous matrix in 4-week old chickens. Likewise, Cole and Wezeman (1985), in the DEF of 5 to 7-dayold mice, describe the presence of a perivascular cellular layer between the wall of the vessels inside the canal and the matrix. Later, Cole and Cole (1989) identified such cells as chondrocytes. The authors did not relate the existence of the above described fibrillar layer either.

We observed the distal segment blind ends of both terminal and perforating cartilage canals as dilations, although Lutfi (1970) describes the lower end of only the perforating canals as a cone. This dilation contains a connective-vascular mass with a glomerular aspect, owing to the abundance of capillary vessels. We have been unable to find any description of such a formation either in chick embryos or in other species, except for the contribution by Wilsman and Van Sickle (1970), where a vascular pattern with a glomerular aspect is described in the endings of some canals in pups younger than 1 week. These authors related the presence of glomerular endings to the appearance of foci of ossification in the surrounding cartilaginous matrix. Gray and Gardner (1969) described the presence of ossification processes in the cartilage canals of human fetal humeri. In our specimens, the vascular glomerulus appeared next to a cartilage tissue with some evidence of cell hypertrophy, although with no signs of ossification or calcification. In this sense, Lutfi (1970) reported that the presence of cartilage canals is not necessarily associated with the appearance of a secondary centre. The secondary ossification centre described in the proximal epiphysis of the tibia (Romanoff, 1960) as the only secondary centre in the avian skeleton, does not appear until the 56th day after hatching (Hogg, 1980). Therefore, the presence of the cartilage canals of the avian epiphyses is not necessarily associated with such centres because the canals do not only appear in the proximal epiphysis of the tibia and, moreover, in this bone they appear sooner than the above-mentioned ossification centre. As previously stated, an obvious limit between the blind end of a canal and the neighboring cartilage is not shown. This observation is more patent at the distal end, where an invasion of the cellular elements from the canal to the adjacent cartilage matrix appears to occur. The paucity of cells seen in the matrix surrounding the terminal end of some canals in our specimens is in agreement with the observations of Watermann (1964), who reported that the invasion of the canals is preceded by the lysis of matrix cartilage cells. This is even compatible with the active chondrolysis is carried out by stellate fibroblast-like cells and macrophages, as described by Chappard et al. (1986) for human fetal cartilaginous epiphyses. However, Delgado-Baeza et al. (1991) did not find any signs of chondrolytic activity in the rat proximal tibial epiphysis cartilage matrix.

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