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Temporary Lobulation in Cartilagenous Models of Long Bones[1]

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ABSTRACT Vascular patterns and the vertical zones of maturing cartilage cells are arranged in geometric radiations. This lobular arrangement reflects a distribution of cells and matrix of differing maturity; those cells and matrix closest to the collagenous wall of the vascularized canal are maintained in an immature state longer than those located toward the periphery of the lobule. This configuration suggests a relationship between the oxygenation of cartilage, its maturity, and its ossification. Arteries in the cartilage canal frequently are destined to become the arteries located within the marrow cavity of mature bones.

The morphology of the vasculature of cartilage suggests an ambiguous vascular-dependent mechanism for calcification and ossification of the cartilage model during the formation of long bones (Bloom and Fawcett, '68). Little morphological evidence has been reported, however, to substantiate the importance of the vasculature in this process. The observations reported here provide support for an indirect role of blood vessels in ossification of long bone models.

MATERIALS AND METHODS

Tibias were removed from New Zealand albino fetal rabbits at daily intervals from the fifteenth day of gestation to thirty-two days (term), and at one and five days neonatal, and were fixed in Karnovsky's fluid, dehydrated through a series of graded alcohols, embedded in paraffin, (60–62°C) and sectioned at 4–6μ. Sections from all specimens received one or a combination of the following stains for connective tissues: Snook's reticulin stain, Masson's trichrome (Rosen, et al., '67), colloidal iron-PAS-Bismarck brown (Townsend, '61), or alcian blue. The resulting sections were studied by bright field microscopy using either non-polarized or polarized light.

OBSERVATIONS

Vascularization consistently appeared within the proximal and distal ends of the cartilagenous model of the tibia long before the beginnings of endochondral ossification. The vessels were located within canals and connected either with vessels in the medullary cavity or with vessels in the perichondrium. The latter entered the metaphyseal or epiphyseal areas of the cartilage model. The boundary of each canal was a connective tissue wall composed of collagenous tissue which was birefringent and stained with aniline blue, silver, and PAS. Peripheral to this collagenous wall, zones of maturing cartilage cells and matrix were arranged radially in zones of maturation (fig. 1). This was suggestive of a lobular configuration in cross section where cells and matrix were arranged in rays of graded maturation. Each ray contained immature cells and matrix adjacent to the canal with cells and matrix of increasing maturity extended peripherally. Staining the territorial matrix with colloidal iron also demonstrated the lacunar size and accentuated the lobular pattern.

The age of cells and the matrix was judged by lacunar size, the distribution of silver stain in the interterritorial matrix, and the amount of territorial matrix which stained with colloidal iron or alcian blue. Silver stains and colloidal iron accentuated the lobular pattern, while Masson's trichrome and hematoxylin and eosin stains tended to obscure it. Areas of ossification and the most mature matrix with large

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lacunae and a thin rim of territorial matrix were located distant to the PAS positive wall of the canals, whereas the least mature matrix in the cartilage was adjacent to the canal where it appeared incompletely organized with polarized light. Parallel bundles of collagen fibers were interrupted at oblique angles by other small fiber bundles. In contrast, the fibers in the matrix at the periphery of the lobule were more organized into parallel bundles of the type seen in lamellar bone. The wall of the canals contained reticular fibers and/or small collagenous fibers. Several small veins within the lumen of the canal were adjacent to the walls of the canal but were not enveloped by PAS positive basement membranes. In contrast, the arteriole, which frequently accompanied these veins, occasionally was PAS positive and stained with reticulin. Nerves were not demonstrated on these vessels but were very evident on the vessels of the marrow cavity. At the epiphysis, in the zone of transition from calcified cartilage to endochondral bone, the cartilage cells which surrounded the cartilagenous canal remained immature even while calcification and the dissolution of more peripheral matrices was occurring (fig. 2, 3). The parenchyma and matrix surrounding the canals displayed lobular patterns of organization. The cartilage in areas corresponding to the zone of proliferation (fig. 4) and the zone of hypertrophy (fig. 5) contained canals as well as did the zone of cartilage calcification (fig. 3). The pattern of lobulation extended radially and also was seen to extend longitudinally as evidenced by a canal cut in oblique section (fig. 6). After calcification of all the cartilage surrounding the canal wall, a spoked structure with radial symmetry remained obvious until further remodeling of the trabeculae occurred to form the marrow cavity. In contrast to the process of calcification and osseoid deposition of the cartilage matrix, extensive remodeling of these structures occurred first in areas adjacent to the vessels where the canals had existed.

DISCUSSION

The maturation of chondrocytes and matrix of fetal or neonatal cartilage models may be related morphologically to the presence of vascularized cartilage canals. Chondrocytes and matrix which are situated at a distance from the canal wall always were more mature than those adjacent to the canals.

The results of this study tend to contradict the suggestion by Wilsman and Van Sickle ('72) that the aged cartilage cells are organized adjacent to the canals. In contrast to their report, the most immature chondrocytes and matrix always were found adjacent to the collagenous walls of the canals, and the progressive stages in the maturation of normal cartilage occurred more peripherally. These data also suggest that the lobule is formed throughout a lengthy course and is thus cylindrical rather than spherical, as was suggested by Wilsman and Van Sickel ('70).

Blood flow through the cartilage may influence the composition of the matrix. While vascularization is not required for development of osseoid tissue from cartilage explants *in vitro* (Crelin and Koch, '65), a change in environment, however, appears to alter the proteoglycans or vary the tendency for cells to produce proteoglycans or collagenous matrix and glycoproteins. Variations in pO_2 in tissue culture have been found to influence the rates of cartilage growth and maturation (Brighton et al., '69); and changes in blood flow also have been reported to affect the growth, maturation and ossification rates in the traditional longitudinal zones of cartilage (Trueta et al., '60). These same parameters could apply to the maturation of cells along the lobular rays.

The vessels which were carried in the cartilage canals may be those of the future marrow cavities of the adult epiphysis and the metaphysis (fig. 2, 3).

An interesting and possible important fact to consider is the consistency with which the most immature chondrocytes are found adjacent to collagenous matrices, for example the cartilage canal wall. The lobules which are arranged around canals are morphological evidence that suggests that the growth and development of cartilage may be influenced by or be an influence on the cartilage canals and their vessels.

PLATE 1

EXPLANATION OF FIGURES

Five day neonatal rabbit tibia: Snook's reticulin stain.

1 Cross section of the cartilage model of the proximal tibia with a cartilage canal demonstrating chondrocytes and matrix arranged in radial lobulation. MC, mature chondrocytes; YC, young chondrocytes. × 460.

2 Area of calcified cartilage and a cartilage canal enveloped by a ring of persistent young cartilage cells. V, vessel. × 282.

3 Remnants of lobular pattern in a calcified cartilage canal. V, vessel; O, osseoid. × 276.

4 Cross section of a cartilage canal from an area corresponding to the zone of cartilage proliferation. Lobulation is evident with young chondrocytes adjacent to the canal (C) and cells of increasing maturity placed periferally. × 284.

5 A canal in cross-section from the zone of hypertrophy. Territorial matrix is unstained by silver. In serial sections stained with colloidal iron, the presence of proteoglycans can be demonstrated where there is no silver precipitated. × 660.

6 An oblique section, showing lobular arrangement of cells along the length of a canal wall. Radial symmetry is prominent. × 400.

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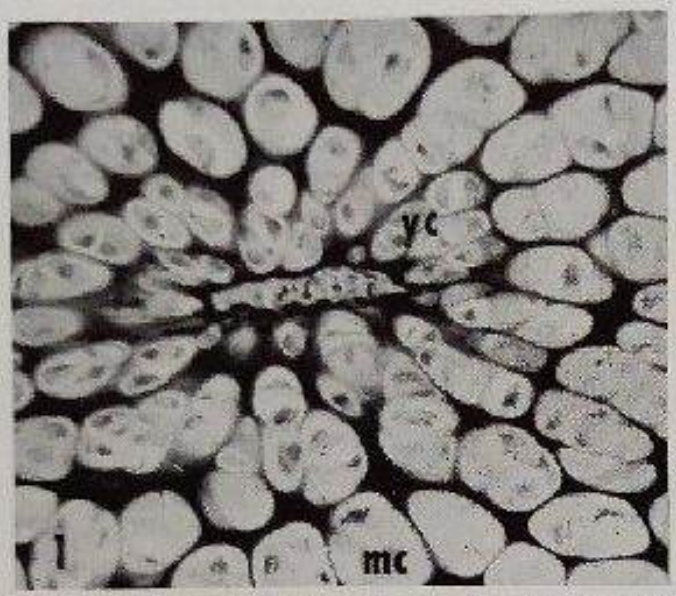
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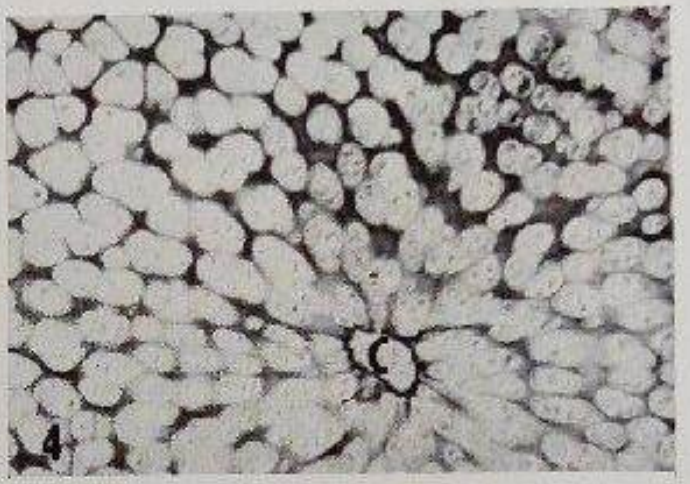
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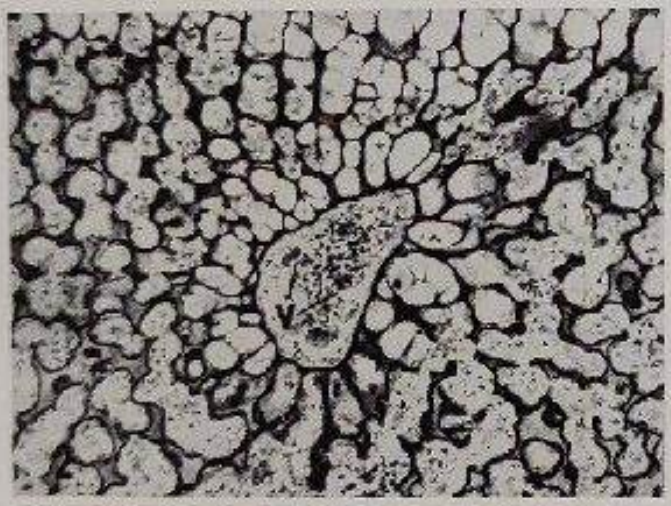
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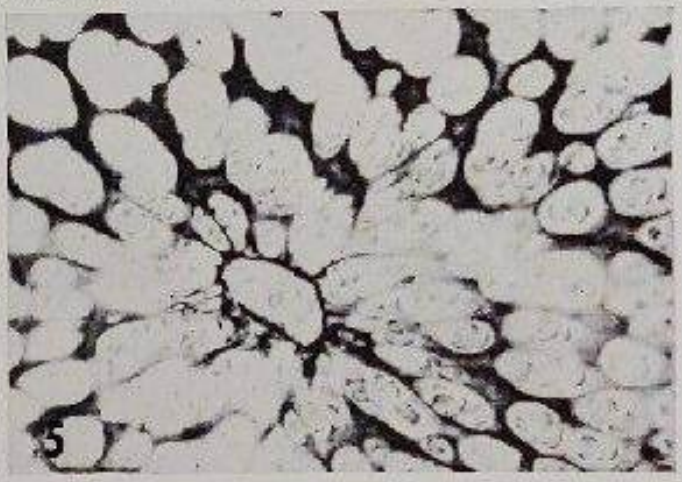
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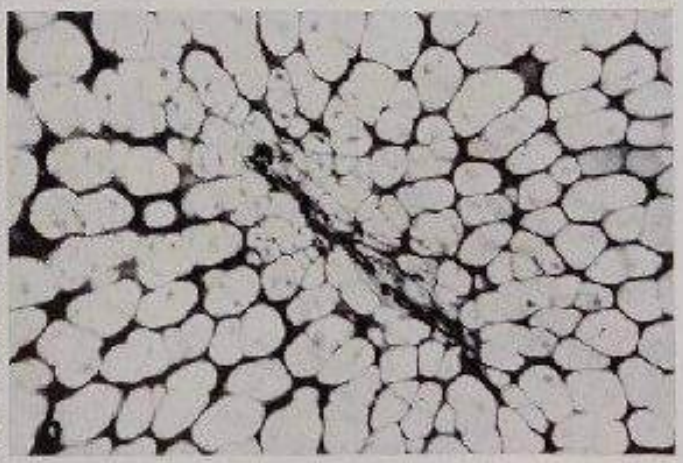
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