Ultrastructural studies on bone lesions of rachitic chicks

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The most characteristic lesions of avian rickets are thickening of the epiphyseal plate, defective calcification of the cartilage matrix, and proliferation of osteoclasts and osteoblasts with an appearance of undifferentiated cells in the metaphysis. However, little attention has been paid to ultrastructure and functional activities of individual cells in the rachitic lesions. Therefore, the pathogenesis of rachitic lesions is still an ill-defined subject. In the present study, the sequential stages of chondrocytic maturation in the epiphyseal plate of normal chicks were examined ultrastructurally with reference to the role of chondrocytes at each stage. Subsequently, the epiphyseal plate, metaphysis and parathyroid gland of rachitic chicks were investigated ultrastructurally and histochemically.

In the epiphyseal plate of normal chicks, the resting zone cells manifested an evidence for active protein synthesis, and the proliferating zone cells had the characteristics of duplicate cells showed by the presence of mitotic activity. The maturating zone cells were rich in cytoplasmic organelles and seemed to synthesize maximum amounts of proteins. Chondrocytes in the calcifying zone were of three distinct types, namely clear cells, stellate cells and hypertrophic clear cells. The appearance of each cell type was closely related to the stage of matrix calcification. Clear cells were observed in the upper calcifying region, where the initial calcification occurred in the matrix. Stellate cells appeared in the middle calcifying region, where further calcification proceeded. Hypertrophic clear cells were recognized in the lower calcifying region, where endochondral ossification was in progress. It was hypothesized that each cell type may play a different role in the initiation, progression, and maintenance of cartilage calcification.

In the epiphyseal plate of rachitic chicks, chondrocytes in resting, proliferating and maturating zones commonly showed a reduction in cell organelles, suggesting a decrease in the protein synthetic activity. In addition, the resting zone cells had numerous intracytoplasmic microfilaments. Mitotic figures were present, but their number in relation to the cell population was decreased. Autolysosome-like dense bodies in the chondrocytes and clusters of degenerative chondrocytes were observed in the proliferating and maturating zones. In calcifying zone of the rachitic epiphyseal plate, initial calcification, characterized by the deposition of apatite crystals in matrix vesicles and formation of spherical crystal clusters, was observed. However, the continuity and coalescence of the crystal clusters was disrupted as a result of insufficient deposition of apatite crystals on collagen fibrils. In this zone, the clear chondrocytes responsible for the initial calcification were observed, while the stellate chondrocytes responsible for the progression of matrix calcification were not detected. The activity of ALPase extended from the lower region of proliferating zone to calcifying zone, this was similar to that in the controls. These findings suggest that the thickening of the epiphyseal plate in rachitic chicks may be caused by the disturbed cell maturation and that the defective cell maturation in the calcifying zone may explain the progressive abnormality of matrix calcification.

In the metaphyseal lesions of rachitic chicks, most of the osteoclasts had detached from the

cartilage and bone surfaces, although they increased in number. Liberated osteoclasts had well-developed cytoplasmic processes and showed normal ACPase activity. Osteoclasts remaining on the cartilage and bone surfaces, although they lacked a ruffled border, were seen phagocytizing bone fragments by cytoplasmic invagination and ingesting spherical calcified nodules by pinocytosis. This finding may suggest an alternative mechanism for bone resorption. ACPase-positive products in these osteoclasts were of greater abundance than those in normal cells. Osteoblasts in rachitic chicks had well-developed RER with dilated cisternae, and the distribution of ALPase- and ACPase-positive products in these cells were the same as in normal osteoblasts. The increased undifferentiated mononuclear cells were divided into two types, round- and spindle-cell types. The former cells were identified as preosteoclasts and the latter ones as preosteoblasts. In the rachitic chicks, hypertrophy and hyperplasia of the parathyroid glandular cells were observed. These cells showed the characteristic morphology of cells at the synthesizing and secretory phases. These findings indicate that hyperparathyroidism may be essential to initiate the proliferation of precursor osteoclastic and osteoblastic cells, which subsequently followed by a corresponding proliferation of osteoclasts and osteoblasts in the metaphysis of rachitic bone.

Original papers of this thesis appeared in "The Anatomical Record", Vol. 242, 29–39 (1995), and "Journal of Comparative Pathology", Vol. 113, 101–111 (1995).

Vitrification of bovine blastocysts derived from *in vitro* maturation, *in vitro* fertilization and *in vitro* culture

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Since the first report of successful cryopreservation of mouse embryos by vitrification (Rall & Fahy, 1985), many studies have focused on the cryopreservation of embryos from other species (Niemann, 1991). However, there has been no report about the vitrification of bovine blastocysts derived from *in vitro* maturation, *in vitro* fertilization and *in vitro* culture (IVMFC). Therefore, the following studies have been carried out to establish efficient procedures for cryopreservation of bovine blastocyst derived from IVMFC by vitrification.

1. Vitrification of IVMFC bovine blastocysts using glycerol and propylene glycol

Four experiments were conducted to determine the optimal conditions for the vitrification of IVMFC bovine blastocysts and to investigate the relationship between survival rates and ultrastructural appearance of the blastocysts after vitrification using the freeze-replica technique. In Exp. 1, the optimal concentrations of glycerol and propylene glycol in the basic medium (modified TCM199) for cooling and warming without formation of ice crystals were determined. A straw containing 0.25 ml of the solution was plunged into liquid nitrogen (LN_2), and then warmed. Vitrification of the medium was observed when both glycerol and propylene glycol were present at the concentrations higher than 45% (v/v) in the solution. In Exp. 2,