Morphological Changes of Epiphyseal Plate in the Long Bone of Chondrodysplastic Dwarfism in Japanese Brown Cattle

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ABSTRACT. A total of 20 cases of disproportionate calves on Japanese Brown cattle were examined morphologically. Investigation of 5 affected calves revealed that the insufficiency of endochondral ossification was confined only to the long bones of the limbs and was not accompanied by other associated changes. On the basis of the histological changes of epiphyseal plate and the affected site, this condition may be called chondrodysplasia of the rhizomelic type. Therefore, this disorder was defined as a bovine dwarfism. From three dimensional image analysis and histological changes of the tibial proximal portion in the other 15 cases, deformity and shortness of the tibia were related to the state of distribution and the degree of damages of the epiphyseal plate. In the affected cartilage matrix, cystoid degeneration, fibrous striation, bone spicule, necrotic foci and rent were found. Inadequate metachromasia suggests the abnormal staining of sulfated glycosaminoglycans and alcian blue stainability may be attributable to the abnormal interactions between proteoglycan and other matrix components. At the interface of cartilage and bone, irregular calcification, fibrotic scar and sealing by osseous tissue with relation to vascularity were found. These changes in this study indicated the failure of modeling on the epiphyseal plate, showing disturbance of chondrocytic differentiation and abnormal formation of the matrix.—KEY WORDS: cartilage, chondrodysplasia, dwarfism, epiphyseal plate, Japanese Brown cattle.

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Dwarfism is reported in various animals including domestic and laboratory animals, and in many cases is related to chondrodysplasia (synonymous with Achondroplasia) [4]. In cattle, incidences of dwarfism are reported in various breeds such as "bulldog calves" [3]. Their phenotypes are shown considerable variation, but the disproportionate type is often found [10, 29]. Although they are often associated with other anomalies, the principal lesion is abnormal endochondral ossification of the long bone of limbs, vertebrae and a part of the cranial base. We recognized the incidence of disproportionate calves in Japanese Brown cattle [16]. In this case, characteristic changes were deformity of the distal or proximal end in the long bone of limbs with shortness and partial disappearance of the epiphyseal plate, resembling that of bovine dwarfism. We consider necessary for pathogenesis to examine pathological changes in anomalous bone in detail. In this study, the following morphological changes in deformative bones were examined: (1) Possible relation of the distribution of epiphyseal plate to the deformity of epiphysis. (2) Histological changes in a thin epiphyseal plate.

MATERIALS AND METHODS

A total of 20 cases of disproportionate calves on Japanese Brown cattle in Kumamoto Prefecture were used in this study. After postmortem examination, many sites of endochondral growth cartilage (sphenooccipital synchondrosis, vertebrae, costchondral junction, iliac crest, long bones of the limbs) and nonskeletal tissuses (liver, pancreas, gastro-intestinal tracts, kidney, urinary bladdar, spleen, heart, lung, brain, pituitary gland, thyroid gland, parathyroid gland and adrenal gland) were histologically examined in 5 cases. For the comparative purposes, two phenotypically normal calves were used.

The proximal portion of the tibia (examined portion) showing obvious deformity from the other 15 cases (Table 1) was cut from the bone shaft at 5 cm and used to following method.

Distribution of the epiphyseal plate: Examined portions were cut into slabs about 5 mm thick parallel to the long axis by a saw. From each bone slab, distribution of the epiphyseal plate were examined by visual inspection. In 3 cases (Nos. 1, 4 and 6), the examined portions were fixed fully in 10% formalin and then decalcified in 5% trichloro-

Table 1. Materials and morphological changes of proximal tibia

Case No.	Age (days)	Sex[b]	Body weight (kg)	Deformity of proximal tibia	Distribution of epiphyseal plate	State of epiphyseal plate		Changes of cartilage matrix of epiphyseal plate			
						Irregular area	Columnar area	Cystoid degeneration	Fibrous striation	Bone spicule	Necrotic lesion
1[a]	4	F	31	++[c]	very few	++	+	+	++	−	−
2	7	M	50	+	few	+	+	+	+	−	−
3	8	F	39	+++	greately few	+++	−	+	++	−	−
4[a]	12	F	44	+	few	+	+	+	+	−	−
5	13	M	35	+	few	+	++	+	+	−	−
6[a]	19	F	38	++	very few	++	±	±	+	−	−
7	44	F	60	++	very few	+	±	+	+	−	+
8	60	F	69	+	few	+	+	+	+	−	++
9	84	M	100	+	few	+	++	+	++	+	+
10	90	F	60	++	very few	++	+	±	+	−	+
11	119	F	95	++	very few	+++	+	+	+	−	++
12	134	F	115	+	few	+	++	+	+	+	−
13	137	F	95	+	few	+	++	++	+	−	++
14	158	F	84	++	very few	++	+	+	+	−	+
15	164	F	88	++	very few	++	+	++	++	−	++

a) Three cases (Nos. 1, 4 and 6) are used to three dimensional reconstruction method.
b) F: female, M: male.
c) −: Negative, ±: slight, +: moderate, ++: marked, +++: more marked.

acetic acid. After decalcification, they were cut into several slabs about 1 cm thick parallel to the long axis. Each slab was embeded in gelatin and frozen by a thermo-freezer (Komatu Electronics Inc.). Serial sections at 1 mm thick were made using a sledge microtome and then photographed. Under certain required conditions [15] , the distribution of the growth plate as a parameter was inputted to the image analyzer (Cosmozone 2SA, Nikon) and the three dimensional reconstruction images were obtained.

Histological method: Bone slabs, which were examined by visual inspection as before, were fixed in 10% neutral buffered formalin. Small blocks from these slabs were degreased in graduated ethanol and decalcified in 5% trichloro-acetic acid for 3 to 7 days. By a routine method, they were embeded in paraffin, cut at 4 to 7 μm and stained with hematoxylin-eosin, azan, van-Gieson and periodic acid schiff reaction (PAS). To examine the proteoglycan of the cartilage matrix, toluidine blue stain with pH value of 4.1 and 2.5 and alcian blue stain mixed with magnesium chloride ($MgCl_2$) with concentrations varying from 0.1 to 1.1 M [23, 24] were used. To determine mineral content of the chondro-osseous interface between epiphyseal plate and metaphysis, samples (approximately 3 mm blocks) including epiphyseal plate were cut from bone slabs fixed in 10% neutral buffered formalin and embeded in paraffin without decalcification. Tissue sections were cut at 10 μm and stained with von Kossa reaction [17].

RESULTS

In affected calves, long bones of the limbs were shortened and deformed at the distal or proximal end without any other consistant lesions. Histologic lesions in the nonskeletal tissues have not been identified in 5 cases. In the epiphyseal plate of the long bones (humerus, ulna, radius, matacarpus, femur, tibia, metatarsus), architectural abnormality with irregular arrangement of chondrocytes and disturbance of cartilage matrix were found and its lesions were varied with the site. Other sites of endochondral growth (sphenooccipital synchondrosis, vertebrae, costchondral junction, iliac crest, articular epiphyseal cartilage of the long bones) were comparable to the controlled calves.

In the tibia, the morphological changes were severe and surmmarized in Table 1. The examined proximal portions of the tibia were concave in shape, because intercondylar eminence had subsided at the center of the articular surface, and internal and external condyles were tall at the medial and lateral edges, respectively. Visual inspection of the bone slabs and three dimensional reconstruction images of the examined portion made clear that the form of epiphyseal plate was similar to the deformity of the articular surface

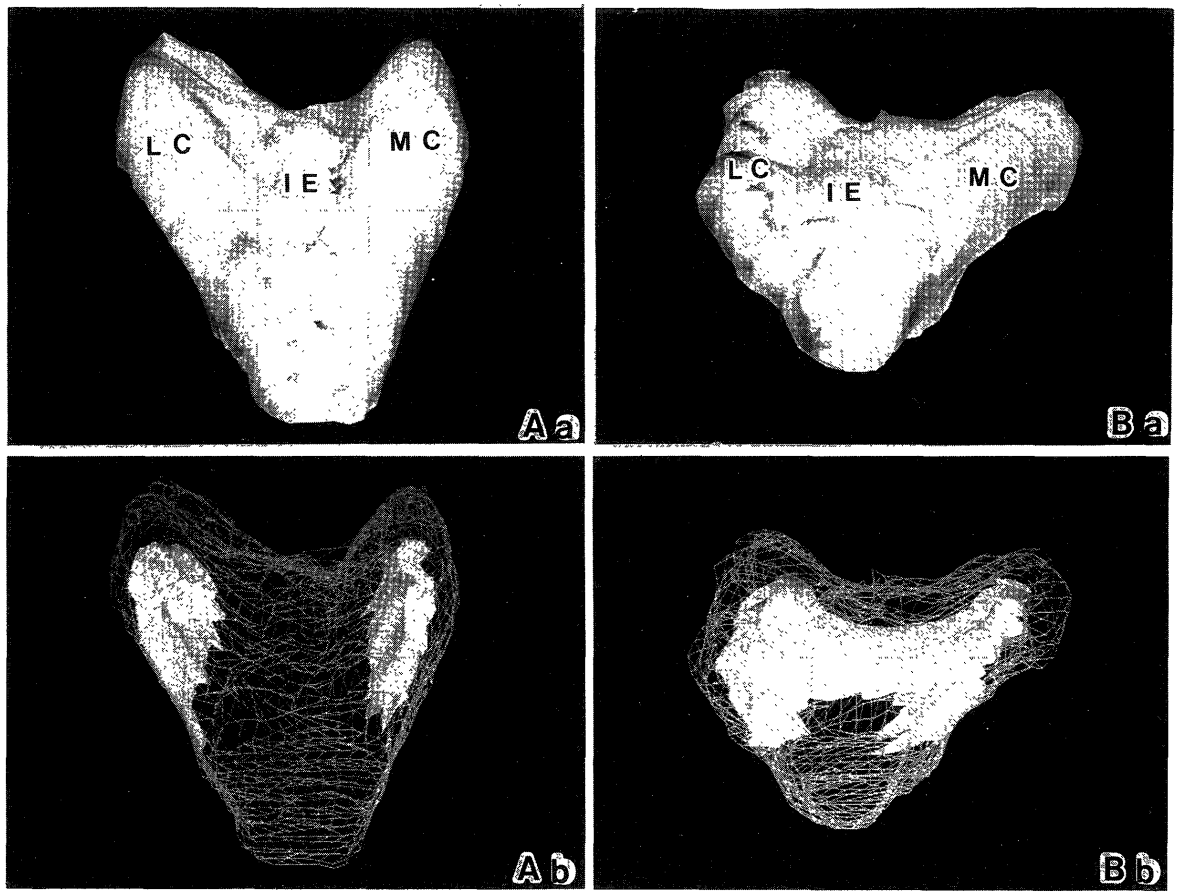

Fig. 1. Three dimensional reconstruction images of examined portion by image analyzer (Cosmozone 2SA, Nikon). Severe deformity of examined portion (A) from case No. 1 and mild one (B) from case No. 4 are shown. Aa, Ba: Surface image shows a front view of examined portion. Ab, Bb: Wireframe image and surface image show the front appearance of examined portion and growth plate, respectively. MC: medial condyle, LC: lateral condyle, IE: intercondylar eminence.

between epiphysis and diaphysis. Some central portions of the epiphyseal plate disappeared, thus indicating the closure of epiphyseal plate in these regions. In the case of lesser concave shape at articular surfaces, the distribution of the epiphyseal plate was greater (Fig. 1). Histologically, the vertical height of the epiphyseal plate was greatly reduced and uneven. The disorganized cartilage tissue protruded from the growth zone papillary or tongue-like into the spongy bone. The cartilage core surrounded by osseous tissue was seen in the spongy bone (Fig. 2). In some cases, the cartilage protrusion extended into compact bone. Histological lesions of the epiphyseal plate were consisted of columnar cell arrangement area prominent in the peripheral portion (columnar area) and irregular cell arrangement area prominent in the central portion (irregular area). The former was indicative of hypoplastic change and the latter of dysplastic change. In the columnar area, each zone boundary was relatively clear and chondrocyte arranged as a palisaded columnar. Many chondrocytes in the zone of reserve cartilage were small, roundish or oval, and a slightly wider matrix stained homogeneously. Slightly larger lunate chondrocytes occupied the zone of cell proliferation, and the population of chondrocytes in the zone of hypertrophy and maturation was greatly decreased (Fig. 3A). In the irregular area, each zone boundary was not clear and chondrocytes with variation in size and shape irregularly arranged. Many chondrocytes were in a state of disorder. Large and vacuolated chondrocytes were often seen, and small acidophilic granules (PAS negative) were present in the cytoplasm (Fig. 3B). In the matrix, the same granules and large eliptical acidophilic deposits were seen, and cystoid degeneration and irregular fibrous striations were prominent beside the less cellular component (Fig.

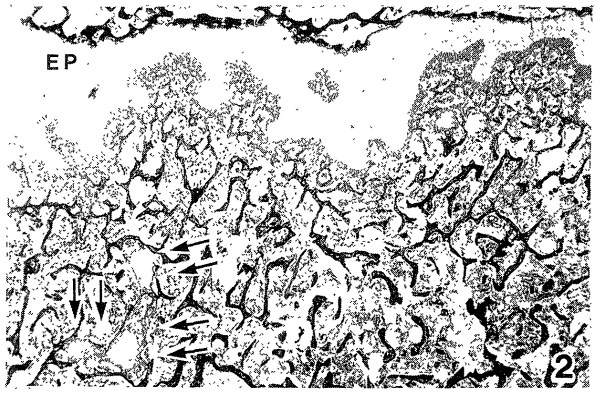

Fig. 2. Morphological changes of epiphyseal plate (case No. 12). Azan stain. × 5. Papillary or tongue-like protrusions of epiphyseal plate (EP) toward metaphysis and cartilage cores surrounded by osseous tissue (arrows) in spongy bone are seen.

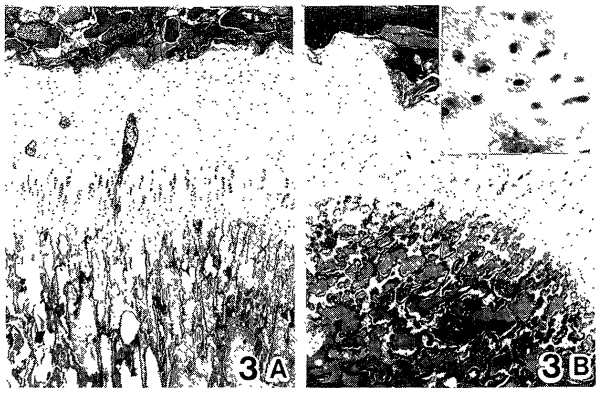

Fig. 3. Histological changes of epiphyseal plate. A: columnar area (case No. 5). HE stain. × 25. Chondrocytes arranged as a palisaded columnar B: irregular area (case No. 7). HE stain. × 25. Irregular arranged chondrocytes are found. Inset: Higher magnification of irregular area. HE stain. × 240. Large and vacuolated chondrocytes, small acidophilic granules in the cytoplasm and in the cartilage matrix are seen.

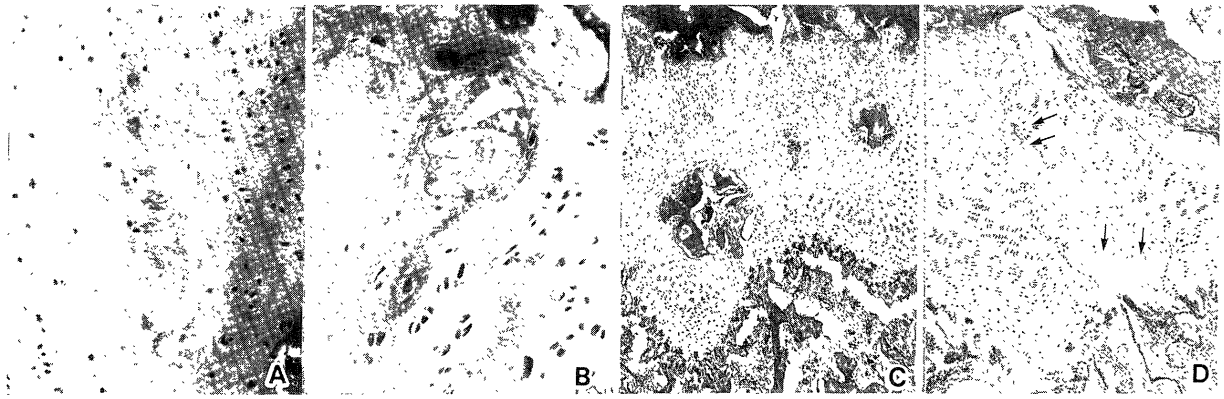

Fig. 4. Changes of cartilage matrix. A: cystoid degeneration (case No. 7). HE stain. × 100. B: irregular fibrous striations (case No. 15). Azan stain. × 110. C: bone spicules surrounded by cartilage tissue (case No. 9). HE stain. × 22. D: necrotic lesion (arrows) and rent (case No. 13). HE stain. × 38.

4A, B). The fibrous area of the matrix was slightly PAS positive and stained blue with azan and red with van-Gieson. Some cases showed bone spicules, necrotic foci and rents in the epiphyseal plates (Fig. 4C, D). These changes were prominent in older and heavier cases (Table 1). The marrow spaces between the trabeculae in the metaphysis were slightly larger than normal and the bony trabeculae were irregularly oriented. No significant differences on the activity of osteoclasts and osteoblasts were observed between the affected claves and the controls. No abnormality were seen in the cortical bone. The irregular vascular ingrowth invaded the epiphyseal plates and the chondrocytes like hypertrophic cells increased in the vascular invaded regions (Fig. 5). At the interface of the cartilage and bone, von Kossa reaction indicated irregular calcification in the interterritorial matrix (Fig. 6). The epiphyseal plate was often sealed by osseous tissue at the papillary and tongue-like protrusions and a closural area without tufts invasion of vessel. The fibrotic scars were also seen at the interface of the cartilage and bone in this region (Fig. 7). In a case (case No. 3), the resorption of cartilage tissue at the conjunction of articular cartilage and compact bone was failed.

With the use of toluidine blue stain, inadequate metachromasia of the matrix were observed in affected calves, which was characterized by unequal metachromasia at pH 4.1 and poor metachromasia at pH 2.5 in the irregular area respectively (Fig. 8). In the controlled calves, metachromasia of the matrix appeared homogeneously in the epiphyseal

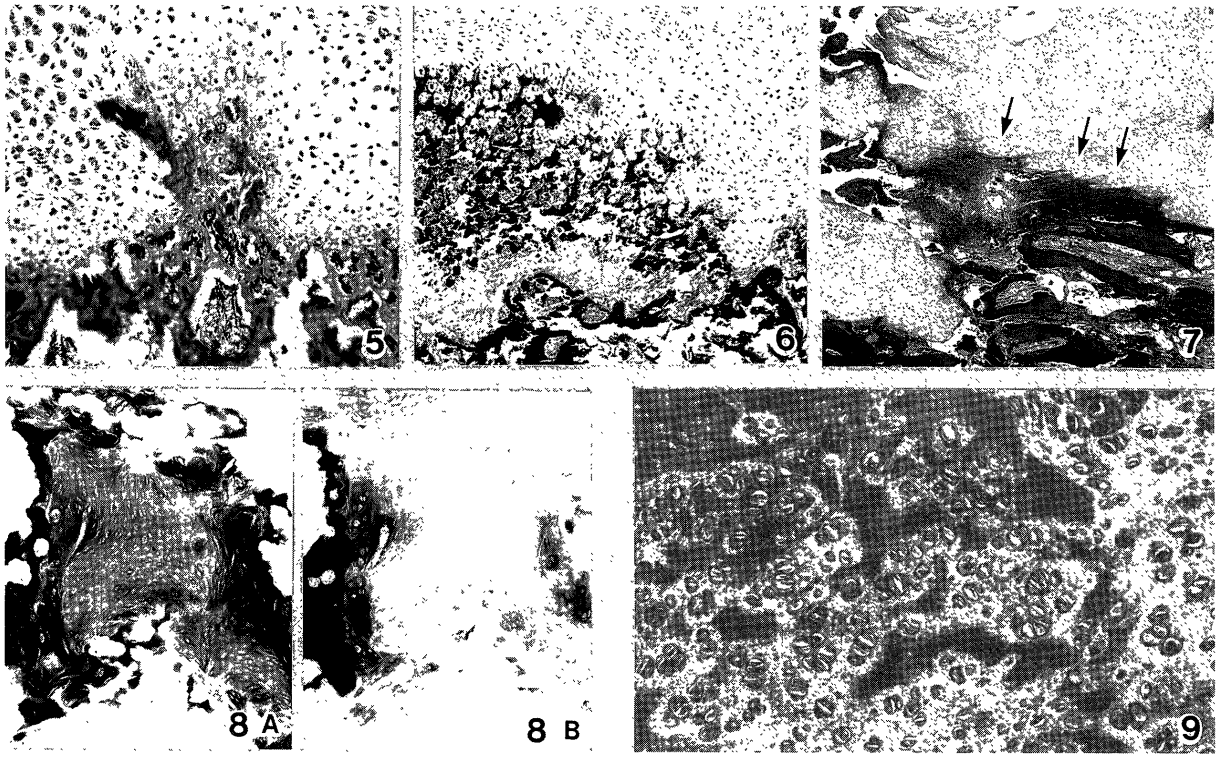

Fig. 5. Irregular tufts invasion of vessel to epiphyseal plate (case No. 10) HE stain. × 56.
Fig. 6. Abnormal calcification at the interface of cartilage and bone (case No. 12). von Kossa stain. × 40.
Fig. 7. Fibrotic scar (arrows) at the interface of cartilage and bone (case No. 9). HE stain. × 10.
Fig. 8. Inadequate metachromasia in the matrix of irregular area (case No. 7). Toluidine blue stain. × 30. Metachromasia of the matrix was unequally at pH 4.1 (A) and poorly at pH 2.5 (B).
Fig. 9. Alcian blue positive materials in the acellular matrix of irregular area (case No. 7). Alcian blue stain with $MgCl_2$ at 0.3 M. × 110.

plate at pH 4.1, while marked decrease in stainability at pH 2.5 occurred in the zone of reserve cartilage. Alcian blue staining added to $MgCl_2$ indicated the interterritorial matrix to be positive up to 0.3 M to 0.5 M at $MgCl_2$ concentration and territorial matrix up to 0.7 M to 0.9 M in the columnar area. In the irregular area, abnormal deposits in the acellular large septa, seen as small granules or large sheet, were very positive up to 0.3 M (Fig. 9) and their alcianophilia gradually decreased at 0.4 to 0.5M and disappeared at over 0.6 M. In contrast, the normal epiphyseal plate retained positive alcian blue reactions up to 0.6 M to 0.8 M in the interterritorial matrix and up to 0.9 M to 1.0 M in the territorial matrix.

DISCUSSION

The lesions of epiphyseal plate examined in this study were histologically characterized by structural abnormality with irregular arrangement of chondrocytes and disturbances of cartilage matrix. These abnormalities suggest the insufficiency of endochondral ossification. Similar changes are also seen in metabolic bone disease (osteopetrosis, osteomalacia and rickets) [9], toxic hypervitaminosis (vitamins A and D) [9], hyena disease [28, 30] and mucopolysaccharidosis [9, 12]. However, these disorders are systemic and characterized by disturbance of bone formation and remodeling due to abnormal activities of osteoblasts and osteoclasts. Furthermore, also seen concomitantly are some morphological changes such as metastatic calcification in toxic hypervitaminosis, accumulation of large foamy cells involved in vitamin A storage in various parenchymatous organs (such as perisinusoidal fat-storing cells in the liver) in hypervitaminosis A, hyperfunctioning of the thyroid gland in hyena disease and cell vacuolation in nonskeletal tissues in mucopolysaccharide storage disease. However, investigation of 5 affected calves in this study revealed that the abnormality of the endochondral growth cartilage

was confined only to the long bones of the limbs and was not accompanied by other associated changes. The disease in these calves was diagnostically found to correspond to chondrodysplasia [9]. It is a defect identifiable at birth and can be classified as defects of growth of tubular bones and/or spine [26]. When classified by affected site (affected part of bone), this condition may be called chondrodysplasia rhizomelic type [27]. Therefore, on the basis of morphological changes, nutritional states and pedigree analysis [16], disproportionate calves on Japanese Brown cattle can be defined as a bovine dwarfism.

In the tibia, the degree of deformity appeared at proximal portion related to distribution of the epiphyseal plate based on the results of three dimensional image analysis and on the visual inspection of bone slabs. From the histological evidence, the columnar area and the irregular area in the same epiphyseal plate indicated the state of hypoplasia and dysplasia respectively. Endochondral ossification would be imbalanced in the irregular area and delayed in the columnar area. The retarded longitudinal growth at the periphery of the epiphyseal plate owing to the predominant columnar area, and no growth at the center of the epiphyseal plate owing to the predominant irregular area possibly caused the concaved appearance at the proximal portion of the tibia. Therefore, while the rate of endochondral ossification appears to be greatly reduced, periosteal ossification is normal, and this relatively increased in rate, resulting in the shortening of bone shaft of the tibia.

The matrix of cartilage showed cystoid degeneration and irregular fibrous striations as the major morphological changes are considered to be degenerative disorder of cartilage with focal death of cells followed by cyst formation, fibrovascular scarring and dystrophic ossification in human diastrophic dwarfism [21]. Acidophilic granules in the cytoplasm of chondrocytes may possibly have been degenerated products differing from glycoprotein seen in human achondroplasia [5, 13] for PAS negative. Fibrous striations of cartilage matrix are regarded as restoration of the erosion of cartilage due to increased collagen fiber [19]. The fibrous striations of cartilage matrix in affected cases appeared related to increased collagen fiber in view of the staining (azan, van-Gieson, PAS). Inadequate metachromasia in the matrix suggests the abnormal staining of sulfated glycosaminoglycans [18]. Materials positive to alcian blue with at a low concentration of $MgCl_2$ were found in human achondroplasia [5] but not in Hereford dwarf cattle [7]. The alcian blue stainability in the matrix, which was characterized by decreased alcianophilia in the columnar area by staining with low concentrations of $MgCl_2$ and deposition of alcian blue positive materials in the irregular area by staining with 0.3 M $MgCl_2$, may be attributable to the abnormal interactions between proteoglycan and other matrix components such as collagen and glycoproteins [6].

At the interface of the cartilage and bone, irregular ossification without cartilage trabecula was recognized at the region occupied by large chondrocytes, in the irregular area. These changes possibly depend on the start of calcification with irregular tufts invasion of vessel following the formation of cell aggregates as in the case of large chondrocytes in some irregular areas. At some papillary and tongue-like protrusion and center of the epiphyseal plate, cartilage tissue was sealed by osseous tissue without vascular invasion, due possibly to fibrous ossification [20] because of fibrous scar at the interface of cartilage and bone in some sealing portions.

From the present study, we conclude that morphologic changes in the epiphyseal plate in affected cases indicate failure of modeling on the epiphyseal plate, due to disturbance of chondrocytic differentiation and abnormal formation of cartilage matrix. In cattle, dwarfism is often reported as achondroplasia and various phenotypes have been found [29] and is also valuable as an animal model [14]. Mild disturbance of the epiphyseal plate is seen in most cases. Similar changes showing normal cell columns in the epiphyseal plate have been found in other mammals (mouse, guinea pig, rabbit) [1, 2, 25]. This is considered due to a premature process of epiphyseal plate closure. The disorganization of the epiphyseal plate in this study which appear to resemble previously described form in lamb [22] and kitten [11] is somewhat severer than the other reported cases.

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