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The Ultrastructure of Osteochondrosis of the Articular-Epiphyseal Cartilage Complex in Growing Swine

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Summary. Osteochondrosis of the articular-epiphyseal cartilage complex (A-E complex) is a significant clinical disease in swine. It has been suggested that osteochondrosis is the underlying cause of osteochondritis dissecans in humans. The purpose of this investigation was to characterize the ultrastructural changes in the earliest macroscopically visible lesion of the epiphyseal cartilage in osteochondrosis of the A-E complex in swine. Osteochondritic epiphyseal cartilage from the distal femora and humeri of growing crossbred boars was collected, embedded in plastic, and studied light and electron microscopically. The predominant lesion was chondronecrosis, characterized by chondrocyte death and loss of matrical proteoglycan. Transition from normal to abnormal cartilage was abrupt. Lipid accumulated in chondrocytes within and adjacent to lesions, but not in chondrocytes distant from lesions. Intracellular lipid accumulation was an important feature of the lesion and may play a role in its initiation. It is hypothesized that intracellular lipid accumulation results from hypoxia/anoxia and may precede matrix degeneration, which precedes cell death.

Key words: Ultrastructure — Cartilage — Osteochondrosis — A-E complex—Osteochondritis dissecans.

Osteochondrosis is a focal failure in endochondral ossification that may result in the formation of an osseocartilaginous flap (osteochondritis dissecans) [1–3]. Etiologies such as trauma, ischemia, and hereditary factors have been proposed [4–10]; however, the cause and pathogenesis of this disease remain unknown. In human beings, necrosis of bone subjacent to articular cartilage is considered by most investigators to be the underlying defect [11–14]. Lesions in cartilage are interpreted to be secondary to the "primary" bone lesion. These observations are based on clinical, radiographic, and surgical findings in patients that have had symptoms of osteochondritis dissecans for extended periods of time, an average of 18 months in one study [9]. Investigations of the early lesions in humans are not available.

Studies of naturally occurring osteochondrosis in animals have concluded that lesions in cartilage precede subchondral bone lesions [2, 3, 15–19]. The earliest macroscopic lesion of osteochondrosis is a focal thickening of epiphyseal cartilage extending into the subchondral bone. By light microscopy, there is decreased calcification and cartilage necrosis, distinguishing this process from nonpathological irregularities in epiphyseal cartilage which are composed of populations of viable chondrocytes [15, 16, 18]. This focal thickening is followed by formation of a fissure extending from subchondral bone to the articular surface. It is following fissure formation and exposure of subchondral bone to synovial fluid that animals become lame and the condition is termed osteochondritis dissecans [20]. At this time, chronic changes may include ossification of the cartilaginous flaps, bone marrow fibrosis, remodeling of trabeculae, and sclerosis of the subchondral bone [3, 17, 20]. These chronic changes are identical to those found in human beings with osteochondritis dissecans [1, 10, 11]. In the early lesions observed in animals, there is no evidence of subchondral bone changes [2, 3, 20].

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It has been suggested that the reported differences in osteochondritis dissecans between human beings and animals are due to the time at which the lesions are examined [20]. In clinical trials with animals, the disease has been studied at various points in time, beginning with a focal area of chondronecrosis observed histologically in the epiphyseal cartilage above the region of proliferating chondrocytes [18], progressing to macroscopic extensions of epiphyseal cartilage into subchondral bone, fissure formation, and subchondral bone changes [2, 3, 15–19]. In human beings, the time from age of onset of symptoms to removal and examination of the osteochondral fragment is usually a period of years [9, 21, 22]; therefore, the disease is always studied in a chronic stage.

We support the hypothesis that osteochondrosis is the same disease in human beings and animals [20] and suggest that swine may be a useful model for understanding the pathogenesis of this disease in humans. Osteochondrosis is a major cause of lameness in swine and occurs with high incidence in young, rapidly growing pigs [2, 3, 23, 24]. Similar to people, the A-E complex site most frequently affected in swine is the medial femoral condyle [13, 15].

In this study, we examined the earliest macroscopic lesion of osteochondrosis in two predilection sites [15, 24, 25] of growing swine. Although previous investigators have described in detail the light microscopic changes in osteochondrosis, there are few reports on the ultrastructure of the lesion [26, 27]. There are no previous reports on the ultrastructure of osteochondrosis at the level of the epiphyseal cartilage of the A-E complex in any species.

Materials and Methods

Twenty newly weaned 3–4-week-old Hampshire-Duroc boars were obtained from one farm. The boars were reared in pens with concrete floors covered with a thin layer of straw or wood shavings and were allowed access to outside concrete yards. Corn-soybean-based rations (with constituents at National Research council-recommended concentration [28]) and water were provided *ad libitum*. Ten boars at 5 months of age and ten boars at 6 months of age were killed with intravenous (IV) injections of barbiturate solution or exsanguination after electrical stunning. All of the animals were clinically normal and no animals were ever lame. Serial 1mm thick slabs of cartilage from the medial femoral condyle and humeral trochlea were collected with an osteotome in a frontal plane. Slabs included the entire thickness of articular and epiphyseal cartilage and a small amount of subchondral epiphyseal bone. Slabs were immediately immersed in 2% purified glutaraldehyde-2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). They were then examined with a dissecting microscope for evidence of increased thickness of cartilage extending into subchondral bone (osteochondritic lesions; Figs. 1, 2). Lesions were dissected from the slab by removing the adjacent normal cartilage and the overlying articular cartilage, with the subchondral bone left intact. Four femoral and four humeral lesions were collected from eight different pigs. These specimens were cut into 1 × 1 × 3 mm blocks and left in primary fixative for one h. After fixation, blocks were rinsed, postfixed in 1% osmium tetroxide-1.5% potassium fer-

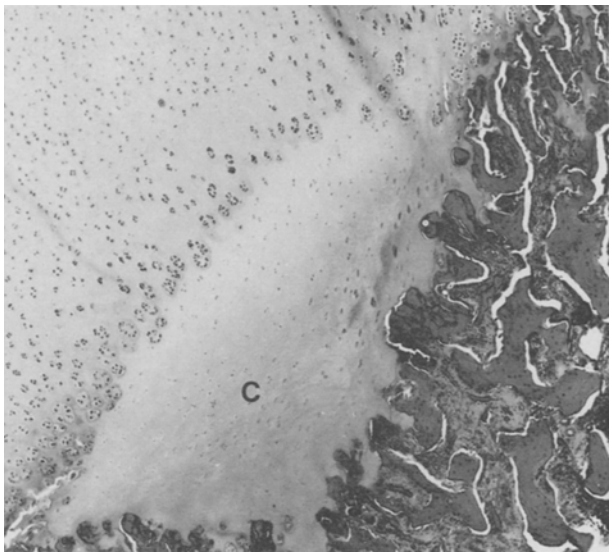

Fig. 1. Early lesion of osteochondrosis in a pig with focal thickening and necrosis of epiphyseal cartilage extending into normal-appearing subchondral bone. Samples for this study were taken from areas of chondronecrosis (*C*) and included adjacent normal epiphyseal cartilage and underlying subchondral bone. H & E (×36).

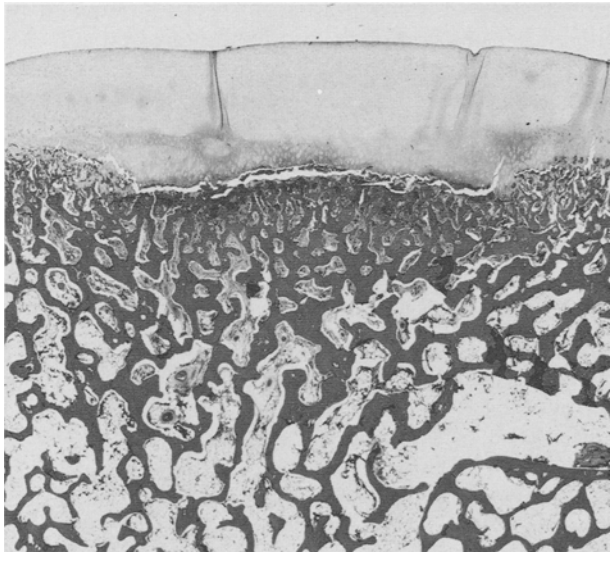

Fig. 2. Chronic lesion of osteochondrosis with thickened epiphyseal cartilage, subchondral bone sclerosis, and cleft formation (osteochondritis dissecans). H & E (×9).

rocyanide [29] for one h, rinsed again, rapidly dehydrated in ascending concentrations of ethanol, cleared in propylene oxide, and embedded in epon. Blocks were sectioned with a diamond knife in a plane perpendicular to the most superficial surface of epiphyseal cartilage. Thick sections, 0.5 μm, were cut and stained for light microscopy [30]. Silver to gray thin sections were cut for electron microscopy. Selected thick (0.5 μm) sections were stained for the presence of lipid [31]. Thin sections were placed on formvar coated 2 mm × 1 mm single slot grids, stained with uranyl acetate-lead citrate, and examined with a Zeiss 10A transmission electron microscope.

Results

Lesions of osteochondrosis collected from femora and humeri were similar morphologically. No differences based on collection site or age were detectable; therefore, cartilage from both sites is described together. Gross, paraffin-embedded histological, and radiographic lesions from these animals have been reported previously [15, 32].

Light Microscopy

The most common light microscopic finding in these samples was populations of necrotic chondrocytes. Seven of the eight samples contained a large population (over half the section) of these cells in one or more blocks. These cells had a darkly staining condensed appearance with little evidence of nuclear or cytoplasmic components other than cytoplasmic lipid (Fig. 3A). Lipid was present in about half of the necrotic cells in any given section and was identified by staining dark blue with a lipid stain [31]. Necrotic cells were surrounded by a wide area of pale staining pericellular matrix giving them a shrunken appearance. Further evidence for cell shrinkage was the fact that many of the cell profiles in a plane of section were missing, leaving only pale, oval-shaped areas of pericellular matrix.

The territorial matrix in the necrotic cell areas was pale staining and had a thready, fibrillar appearance, as opposed to the smooth, homogeneous appearance of normal matrix. (Blocks containing these areas presented sectioning difficulties, as the tissue was prone to compression and tearing.) Populations of dead chondrocytes were found in all of the maturation regions (resting, proliferating, hypertrophic, or calcifying).

Also found within these samples were populations of chondrocytes which were free of morphological abnormalities. The transition from normal-appearing to abnormal-appearing cartilage was usually abrupt, with morphologically normal cells and matrix immediately adjacent to necrotic cells and fibrillar matrix. The transitional cells bordering the lesion area often contained large amounts of lipid as compared with chondrocytes further from the lesion; however, the greatest amount of lipid was seen in necrotic chondrocytes. Occasionally, there was a more gradual transition between "normal" and abnormal cartilage. Here, cells closest to the area of chondronecrosis had wide halos of pericellular matrix, the width decreasing around cells progressively further from the lesion.

In lesions that included the hypertrophic and calcifying cell regions there were chondrocyte clusters (groups of greater than 20 cells with normal mid-hypertrophic cell morphology in healthy matrix) bordering necrotic cell areas. The chondro-osseous junction in these lesions was characterized by an abrupt transition from noncalcified to calcified matrix, with an apparent decrease in the number of invading capillaries. In lesions involving the resting and proliferating cell regions, the chondro-osseous junction appeared normal. No evidence of osteonecrosis was observed in any of the sections.

One lesion area out of the eight sampled contained a large population of vacuolated cells adjacent to the dead chondrocytes. These cells appeared viable at the light microscopic level but contained numerous vacuoles which sometimes exceeded half the cell area in a plane of section.

Electron Microscopy

Cells identified as dead cells at the light microscopic level lacked cell membranes, nuclei, or cytoplasmic organelles, and appeared as electron dense, irregularly shaped aggregates. Large droplets of lipid were common and sometimes exceeded half the cell area in a plane of section (Fig. 3B). The pericellular matrix size was usually much larger than that adjacent to chondrocytes free of morphological lesions and was composed of fine filamentous material, generally lacking collagen fibrils and sometimes containing necrotic cell debris (Fig. 3C). Irregularly shaped, nonmembrane-bound electron-dense aggregates observed in the pericellular matrix of some late hypertrophic and calcifying zone cells in normal epiphyseal cartilage [29, 33, 34] were absent in necrotic areas and rare in the more normal appearing cartilage collected from lesion areas.

Territorial matrix in necrotic cell areas lacked the majority of the proteoglycan components, with only collagen fibrils and a small amount of proteoglycan remaining (Figs. 4A, B). There was no evidence of

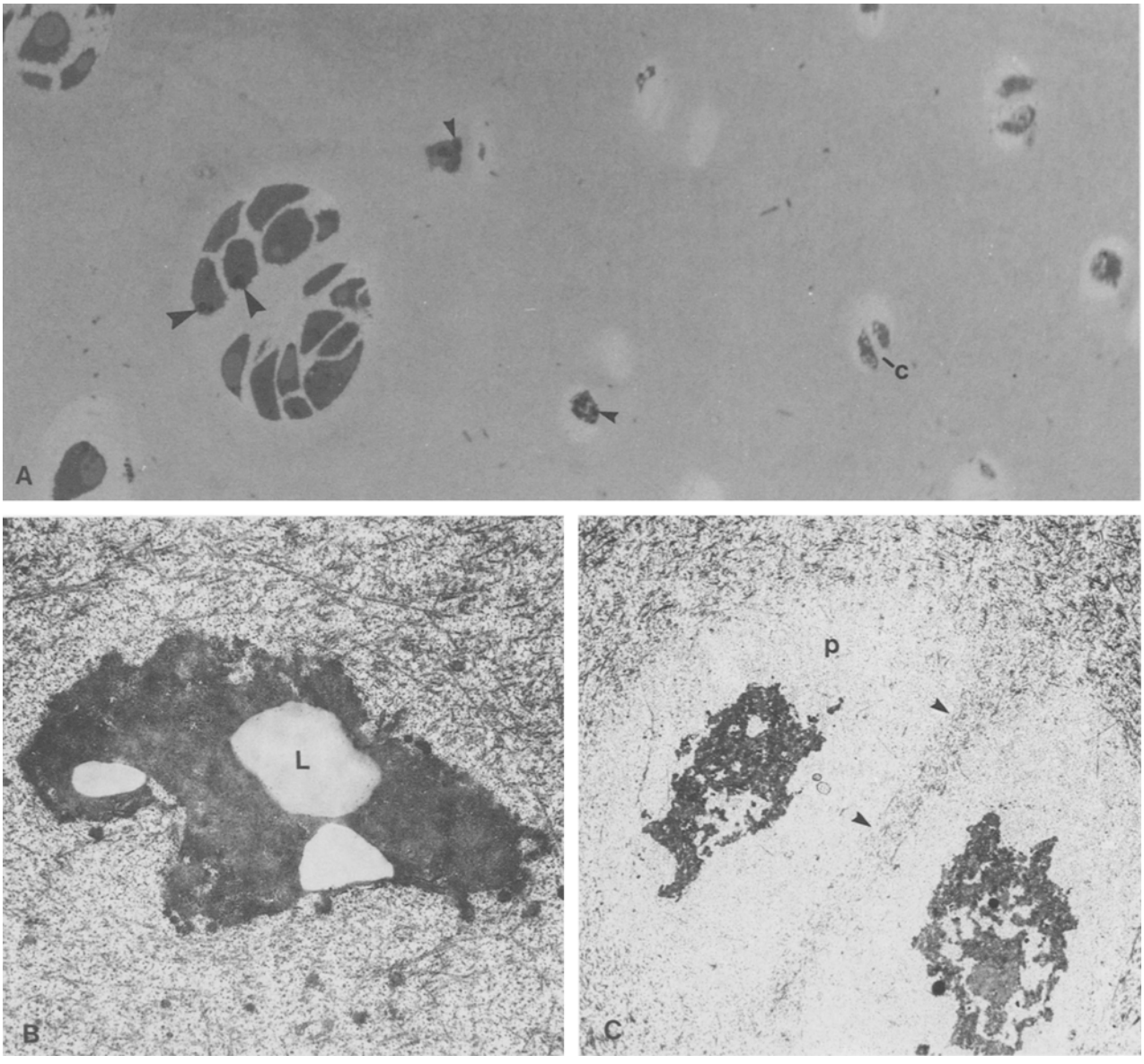

Fig. 3A. Hypertrophic zone of cartilage in a pig with osteochondrosis with an abrupt transition from normal chondrocytes (left) to an area of chondronecrosis (right). Necrotic cells lack recognizable cell structure and are surrounded by a wide area of pale-staining pericellular matrix (*c*). Dark staining intracellular lipid is present in both normal (*large arrowheads*) and necrotic (*small arrowheads*) cells. Methylene blue and Azure-2 stain (×680). **B.** Electron micrograph of a necrotic chondrocyte similar to those indicated by small arrows in A. This cell contains lipid (*L*) and lacks cell membrane or organelles (×16,375). **C.** Pair of necrotic chondrocytes similar to those indicated by (*c*) in **A.** These cell remnant profiles lack lipid and possess a wide area of pericellular matrix (*p*) separated by a narrow band of coarse, fibrillar territorial matrix (*arrowheads*) (×4750).

matrix calcification in necrotic cell areas, nor was there evidence of extracellular lipid.

Chondrocytes directly bordering populations of necrotic cells had normal morphology other than increased amounts of large lipid droplets. Territorial matrix adjacent to these cells was distinctly more coarse than what is considered normal [33].

Chondrocytes forming cell clusters (>20 cells in a plane of section) had normal ultrastructural morphology and were uniformly in a mid-hypertrophic stage of development. These cells had a large amount of rough endoplasmic reticulum. Collagen fibrils formed a rim around these cell clusters. Directly adjacent to these were necrotic cell areas with matrix lacking the proteoglycan component (Fig. 5). No detailed evaluation of collagen was included in this study; however, it appeared that fibril diameter was smaller than usual in lesion areas.

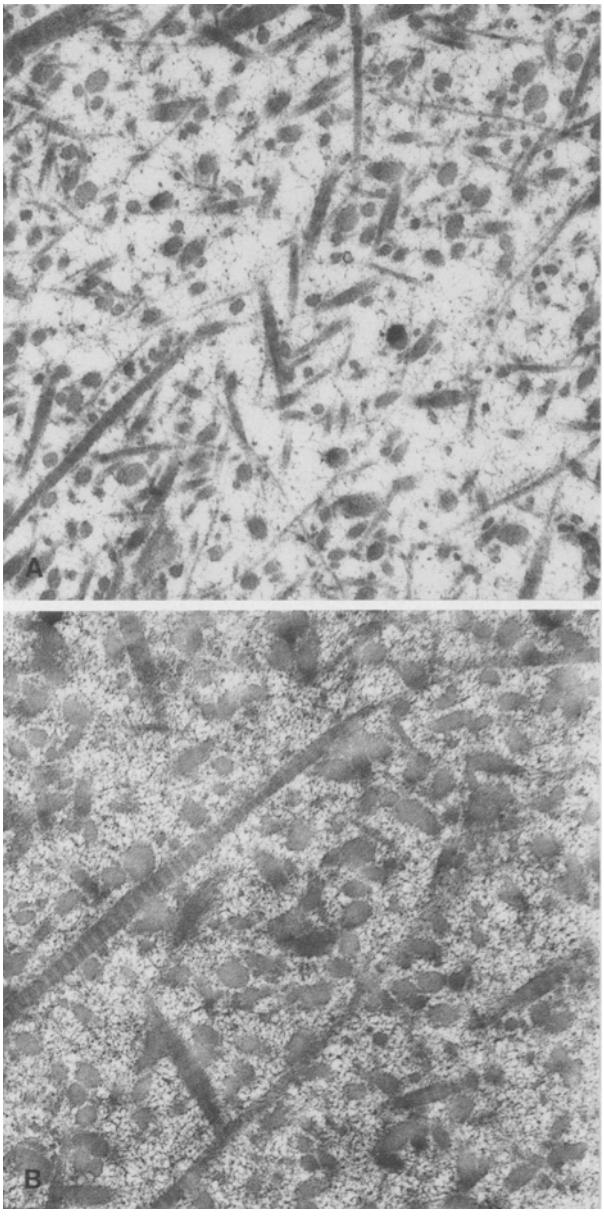

Fig. 4. Electron micrographs of territorial matrix (×37,000). **A.** Lesion area with decreased matrical proteoglycan. **B.** Normal.

Vacuolated cells found in one lesion lacked pericellular matrix. Collagen fibrils characteristic of territorial matrix were located directly adjacent to the cell membrane. Vacuoles occupied the majority of the cross-sectional area of the cell and some contained electron dense aggregates as well as fine strands of material. They did not distort the cell to the degree that cell processes were lost (Fig. 6).

Discussion

The most significant findings in this study of osteochondrosis of the A-E complex were intracellular lipid accumulation, matrix degeneration, and chondronecrosis. Excessive lipid within viable chondrocytes represented the most consistent cellular evidence of proximity to an area of chondronecrosis. Although lipid is considered a normal and constant

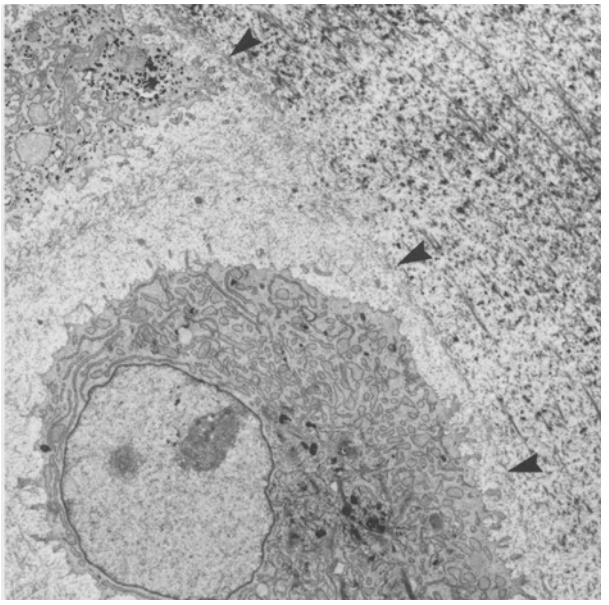

Fig. 5. Cells at the edge of a cell cluster that bordered a lesion. These cells have normal mid-hypertrophic morphology. Fibrils form a rim around these cells (*arrowheads*) marking the transition to abnormal matrix (×4330).

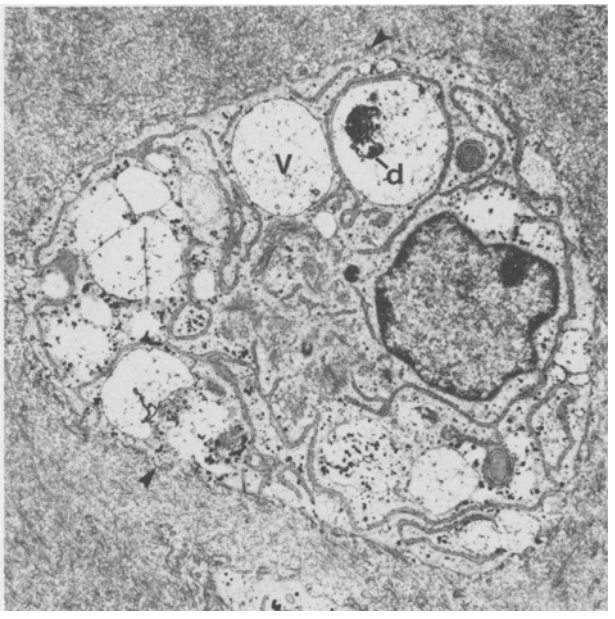

Fig. 6. Electron micrograph of a vacuolated cell from a lesion. Vacuoles (*V*) may contain dense material (*d*). Cell processes are present (*arrowheads*) (×7090).

inclusion in chondrocytes [35–39], lipid accumulation has been observed to be one of the most frequent changes found in degenerating chondrocytes [40]. Intracellular lipid accumulation has been observed in several human chondrodysplasias [41–46]. Stanescu et al. [47] have described a possible hereditary disorder in people that produces an accumulation of complex lipids in articular chondrocytes along with precocious arthrosis. Although lipid accumulation may represent a nonspecific degenerative change in cartilage, several studies on articular cartilage indicate a possible role for lipid in the initiation of chondrocyte degeneration. Bonner et al. [35] noticed a progressive intracellular lipid accumulation with age in articular chondrocytes in human beings and suggested a pathological significance correlating the area of intracellular lipid accumulation with the area in which the earliest lesions of osteoarthritis appear. Stockwell et al. [48] reported an increased amount of intracellular lipid with subsequent cell death in articular cartilage following experimental severance of the anterior cruciate ligament in dogs. Intraarticular injection of lipid into rabbits leads to subsequent uptake of lipid by chondrocytes, loss of glycosaminoglycans from the matrix, and chondrocyte degeneration [49–51]. The conclusion drawn from these studies is that lipoarthrosis produces pathological changes in chondrocytes. Hypothesized mechanisms include a membranolytic effect of oleic acid in triglycerides containing this acid [50] or an alteration in chondrocyte metabolism, possibly caused by the uptake of abnormally large amounts of fat and reesterification of fatty acids [49].

Intracellular lipid increased in amount within lesions, as well as in chondrocytes adjacent to lesions. The source of the excess lipid in A-E complex lesions of osteochondrosis is not known (increased synthesis vs. increased supply of precursors), nor is the sequence of events leading up to this accumulation known. In a study of osteochondrosis in dogs, an increase in intracellular as well as matrical lipid was demonstrated by histochemical staining in areas with lesions, as compared with normal cartilage [52]. This increase was interpreted to result from a metabolic response of the chondrocyte to an altered microenvironment. We speculate that intracellular lipid accumulation precedes matrix degeneration which precedes cell death. Following these events a fissure forms in the necrotic cartilage, leading to changes in subchondral bone including bone marrow fibrosis, remodeling of trabeculae, and sclerosis. The cartilage flap may ossify as a chronic sequela if provided with a blood supply.

Matrical degeneration and chondronecrosis were consistent lesions in this study. These changes have been observed previously in osteochondrosis of the porcine A-E complex [7, 21–23]; however, these reports did not include electron microscopy. It is evident from this study that matrical proteoglycan is diminished in lesions. The proteoglycan component of cartilage matrix is correlated with compressive strength [53, 54], possibly stabilizing the interaction between collagen and chondronectin [55]. Loss of this component leads to an increased mechanical stress on chondrocytes [56–58]. We hypothesize that, subsequent to proteoglycan loss from the matrix, the chondrocytes degenerate. Following these events, trauma may cause cartilaginous flap formation.

The mechanism of proteoglycan degradation is unknown but it must occur extracellularly [55]. The destruction is probably due to enzymes either in cartilage or synovial fluid [59–60]. *In vitro* experiments demonstrate the dependence of matrix degradation on live chondrocytes [61–63]. If this is true *in vivo,* matrix degradation must precede chondrocyte death. The mechanism of lesion repair appears to involve cell clusters with accelerated synthesis of matrix components [15]. The capability of this tissue to repair is related to the severity of the lesion and the subsequent stress placed upon it.

The abrupt transition from normal cells and matrix to areas of chondronecrosis is compatible with a vascular insult. Experimentally, lesions grossly and histologically similar to osteochondrosis have been reproduced in animals by surgical blockage of vasculature [64–66]. In addition, recent histological evidence associates cartilage canals which do not appear to be functional with areas of chondronecrosis in spontaneous A-E complex lesions in swine [18]. We hypothesize that the underlying condition altering the environment of chondrocytes in osteochondrosis is hypoxia/anoxia due to decreased blood supply. This hypoxic environment leads to intracellular lipid accumulation and matrix and chondrocyte degeneration. The cause of the failure in vascular supply is not known at this time. Trauma may aggravate the condition, leading to osteochondritis dissecans.

The vacuolated cells observed in one lesion in this study are similar to those observed in some of the human mucopolysaccharidoses [67, 68]; however, the vacuolization observed in our study is less severe and does not distort the chondrocytes to a comparable degree. As in the human mucopolysaccharidoses, the appearance of these vacuoles is consistent with that of altered lysosomes. The significance of this finding is unknown. It is possible

that vacuolization is a nonspecific lesion of chondrodysplasias.

In summary, we were able to study the early lesions of osteochondrosis in young pigs prior to the onset of cleft formation or lameness. At this time, there were focal areas of thickened epiphyseal cartilage, containing chondronecrosis and matrix degeneration, extending into subchondral bone. Intracellular lipid accumulation was a prominent feature of chondrocytes within and adjacent to lesions, but not distant from lesions. Intracellular lipid accumulation is a feature of degenerating chondrocytes and may be an important factor in the pathogenesis of osteochondrosis. A better understanding of osteochondrosis in animals should lend insight into the pathogenesis of the disease in human beings.

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