Avian Tibial Dyschondroplasia

I. Ultrastructure

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Tibial dyschondroplasia is an abnormality of the growth cartilage that occurs in chickens and other rapidly growing animals. The disease is characterized by a mass of avascular opaque cartilage, which is continuous with the growth plate of the proximal tibia and extends into the metaphysis. In this study electron micrographs revealed that chondrocytes in the hypertrophic zone of the growth plate were normal in appearance with the exception that the cells did not undergo complete hypertrophy. In the proximal region of the lesion, cells began to undergo necrotic changes suggestive of an energy depletion. These changes included dilatation and vesiculation of the endoplasmic reticulum, enlargement of the paranuclear space, mitochondrial swelling with dilatation of the intracristal spaces and the appearance of electron-dense, flocculent material in the mitochondrial matrix, chromatin margination, and dilatation of the Golgi saccules. Chondrocytes also occurred with rarefied cytoplasm and atrophic Golgi saccules. A few cartilage cells in the proximal region of smaller lesions contained crescentic caps of condensed chromatin in the nuclei, which is indicative of apoptosis. These cells also exhibited dilated endoplasmic reticulum and lamellar bodies; and sometimes, in the proximal region of the lesion, they appeared to be condensed and convoluted. This process continued in the mid and distal regions. The condensed necrotic cells appeared as amorphous osmiophilic masses with karyorrhexic and pyknotic nuclei. Matrix vesicles were observed at all levels of the lesion, but calcified only at the distal edge of the lesion, where mineralization of both matrix and cells occurred. The resulting shell of mineral may act as a diffusion barrier. (Am J Pathol 1985, 119:175–190)

TIBIAL DYSCHONDROPLASIA (TD) is characterized by an abnormal plug of nonvascularized, unmineralized, white opaque cartilage dominating the proximal metaphysis of the tibiotarsus and occasionally the tarsometatarsus in chicks (see Figure 1). Leach and Nesheim[1] were the first to report this condition and demonstrated that its occurrence was influenced by diet as well as genetic susceptibility. While TD occurs on diets adequate in all known nutrients, incidence can be altered by changes in the electrolyte balance and the calcium/phosphorus ratio. The effects of diet upon the incidence do not appear to be mediated via changes in blood chemistry. Other terms used in the literature to identify this abnormality are "osteochondrodystrophy,"[2] "focal osteodystrophy,"[3] and "cartilage plug."[4] TD may be similar to a generalized cartilage defect called osteochondrosis,[5] which occurs in mammals. Osteochondrosis has been reported in rapidly growing animals such as dogs,[6] horses,[7] and pigs.[8,9] A defect in endochondral ossification which occurs in humans, called metaphyseal chondroplasia, bears a number of similarities.[10]

The TD lesion gradually regresses with increasing age. Degeneration of the cartilage plug with subsequent normal ossification occurs at about 12–14 weeks of age in the chick.[11] The tibiotarsus and other affected bones remain severely malformed.[12] The gross appearance of the lesion is also similar to that of a lesion which occurs with copper deficiency; however, chickens affected with TD do not exhibit other symptoms associated with copper deficiency.[1] Further studies did not reveal defects in copper metabolism associated with the development of the abnormal cartilage.[13]

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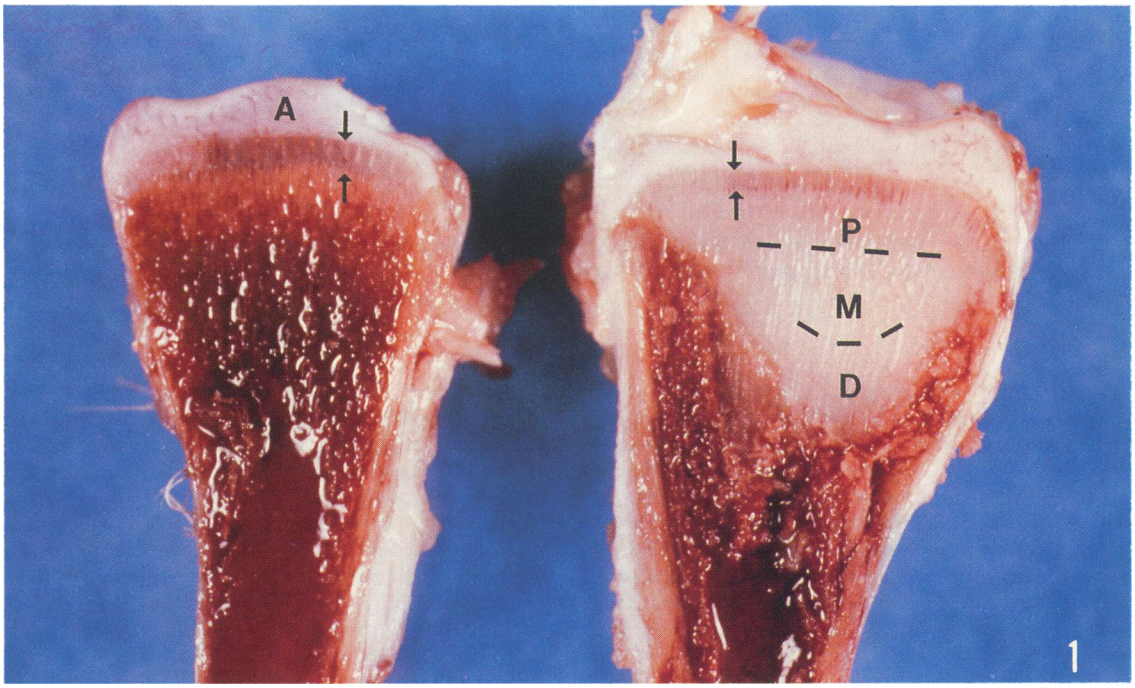


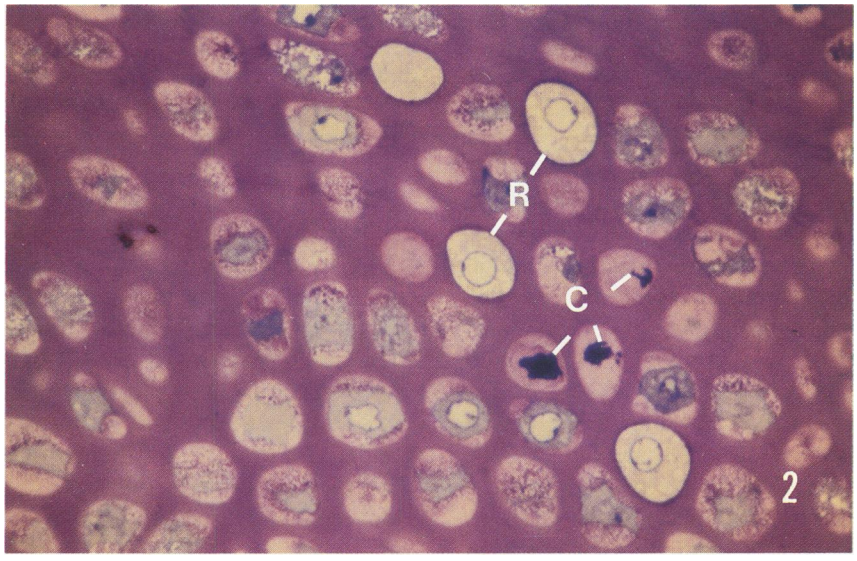


Figure 1—*Left*, The normal proximal tibiotarsus showing the epiphyseal growth plate (*between arrows*) and the articular cartilage (*A*). The epiphyseal plate appears as a thin layer of translucent cartilage with numerous capillary tunnels extending from the epiphyseal arteries. The metaphysis is characterized by numerous trabeculas separated by the metaphyseal vessels which ascend the shaft of the bone to the growth plate. *Right*, A severe dyschondroplastic lesion in the proximal tibiotarsus. The lesion, an opaque cartilaginous mass, lies beneath the growth plate (*between arrows*) and displaces the spongy bone of the metaphysis. The trabeculas extending from the spongy bone appear normal in size but lack the parallel arrangement observed in the normal metaphysis. Regions of the lesion: *P*, proximal; *M*, mid; *D*, distal. (×3) **Figure 2**—Light micrograph of the proximal region of the tibial dyschondroplastic lesion. Rarefied cells (*R*) completely fill their lacunas and are characterized by disrupted and vacuolated cytoplasm and enlarged nuclei with marginated chromatin. Condensed chondrocytes appear as a dense convoluted mass (*C*). (Toluidine blue [1%], ×535)

The lesion is characterized by an accumulation of chondrocytes in the metaphysis. These cells do not stain like normal hypertrophic chondrocytes when treated with alcian blue–PAS.[1] Few studies have been made of the morphology of the TD lesion at the ultrastructural level. Siller[12] reported that the bulk of the TD lesion consists of condensed autolytic cells but did not describe ultrastructural changes which precede the condensation of the cells into autolytic masses. The ultrastructure of normal avian epiphyseal plate has been described by Howlett.[14]

A morphologic study of the ultrastructure of the TD lesion is important in elucidating the mechanisms of abnormal endochondral ossification. This becomes especially true in light of the fact that few communications have been made regarding ultrastructural changes associated with skeletal disorders.[15,16] An advantage in using TD as a model is the low cost and ease of developing large numbers of chickens with this abnormality.

The goal of this investigation was to study the ultrastructural changes associated with the matrix and chondrocytes as one proceeds from the early hypertrophic zone of the TD growth plate to the distal region of the lesion. The pathologic changes in the tissue may provide insight into the etiology of this skeletal dysplasia and may also help to establish a model for the process of abnormal endochondral ossification. Such a model may lead to preventive measures for combating the skeletal dysplasias in humans and other animals.

Materials and Methods

Epiphyseal plates and dyschondroplastic lesions were obtained from 25-day-old chicks of the Hubbard × Hubbard strain maintained at The Pennsylvania State University Poultry Farm. The diet contained the following ingredients (grams per 100 g body weight): glucose, 37.36; casein, 10; ground corn, 31.2; soybean meal, 8; gelatin, 3; wheat gluten, 4; DL-methionine, 0.17; mineral mixture,[1] 5.05; and vitamin mixture,[1] 1.22. Each region of the lesion (ie, proximal, mid, and distal) was examined in tissue from 4–5 birds.

Chicks were killed by cervical dislocation, and the growth plates and associated lesions of the proximal tibia were quickly exposed. Thin slices of tissue were dissected into 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2, 0 C, 3 hours), washed in buffer, postfixed in 1% OsO_4 in 0.1 M cacodylate buffer, dehydrated with ethanol, and embedded in Spurr's resin.

Tissue slices were also prepared by simultaneous glutaraldehyde and OsO_4 fixation[17] and uranyl acetate *en bloc* staining.[18] Thin sections were cut at a thickness of about 700 Å and stained with a saturated solution of uranyl acetate in 70% ethanol followed by Reynolds' lead citrate.

Results

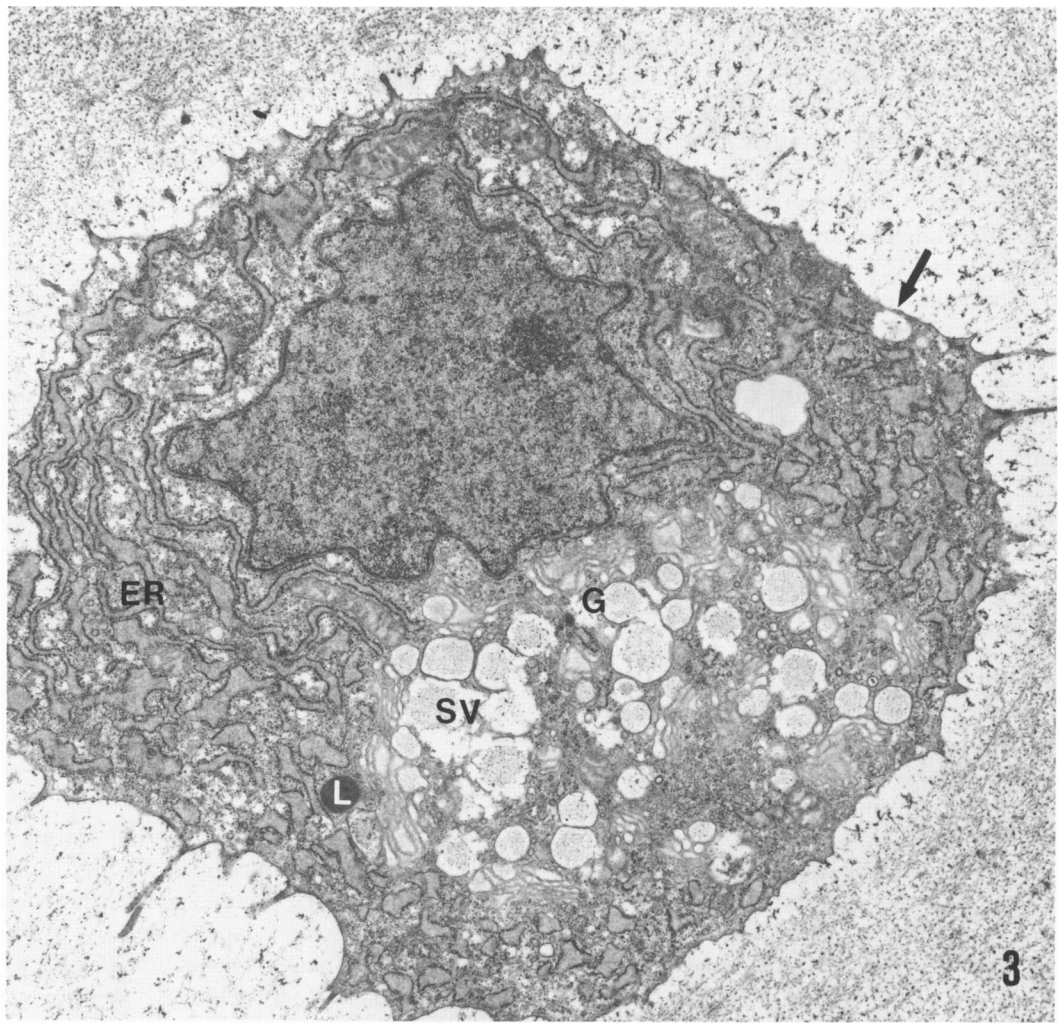
Mediolateral sagittal cross-sections of the proximal head of the normal tibiotarsus and a similar cross-section from an afflicted chick are shown in Figure 1. The abnormal epiphysis is enlarged and contains a cartilaginous plug which extends from the growth plate to a layer of spongelike bone.

The chondrocytes in the proximal region of the TD lesion, immediately distal to the TD growth plate, are polymorphic, some being condensed, others rarefied, and still others normal in appearance (Figure 2). The polymorphic appearance of the proximal region of the lesion distinguishes it from the mid region and early hypertrophic zone of the growth plate. The hypertrophic cells appear normal, but the mid region of the severe lesion is characterized by the presence of numerous condensed chondrocytes, which appear as small, dense-staining bodies.

Early Hypertrophic Zone

The chondrocytes in the early hypertrophic zone usually appeared to be undergoing hypertrophy in a normal fashion and exhibited normal morphologic features. They were characterized by well-developed granular endoplasmic reticulum (GER), Golgi complexes, and secretory vacuoles (Figure 3). The cells were up to 20 μ in diameter, were approximately spheroid, and contained many slender cytoplasmic extensions. The nuclei, which were located eccentrically, had an undulated appearance. Fine chromatin granules were dispersed evenly among the euchromatin and nuclear sap. Nuclear pores were occasionally observed along the nuclear envelope. Electron-dense, lysosomelike bodies, approximately 0.5 μ in diameter, were present in many cells of the early hypertrophic zone. The endoplasmic reticulum was usually ribbon-shaped, with some dilated portions bordering the regions which contained the Golgi complex and secretory vacuoles. The cisternae of the GER were filled with a moderately electron-dense medium.

In early hypertrophic chondrocytes, which are active in secreting the extracellular matrix components, the Golgi-secretory region contained large scalloped-edged vacuoles approximately 300–600 nm in diameter (Figure 4). These secretory vacuoles were bound by a smooth membrane and contained a finely granular and filamentous material which resembled matrix granules. Moderate-sized secretory vacuoles approximately 250–300 nm in diameter were also observed. They ap-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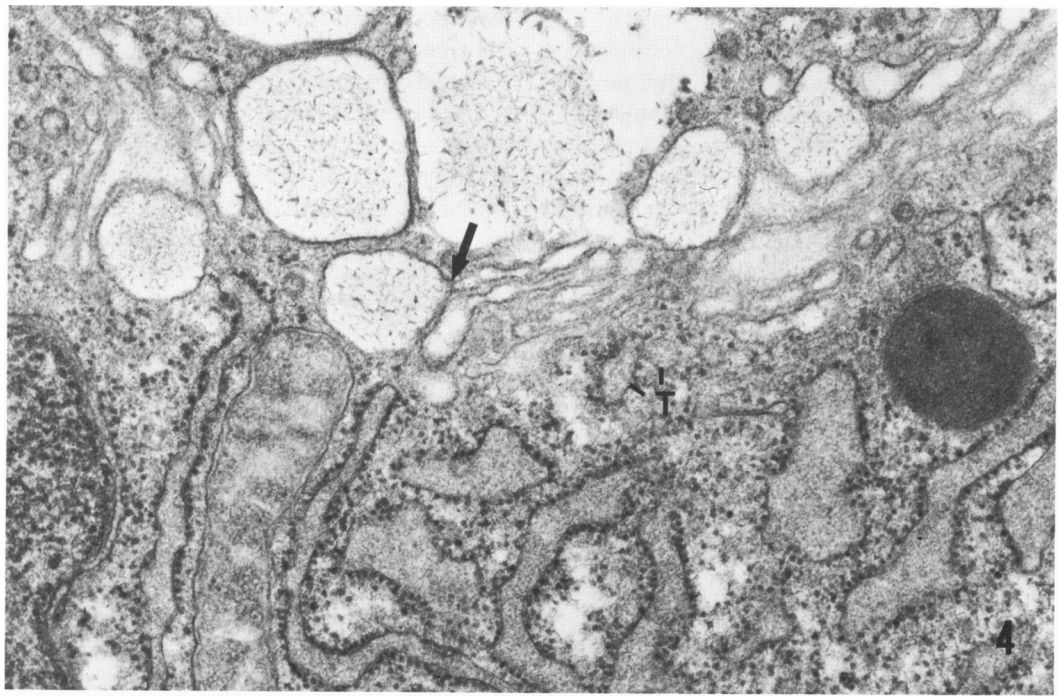
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peared to be continuous with the Golgi saccules and may represent the swollen ends of the sacs. These vacuoles, like the larger vacuoles, contained a fine fibrillar material. Small coated vesicles were observed among the Golgi saccules and secretory vacuoles. These small vesicles, 50–100 nm in diameter, had bristles on their cytoplasmic surface. They appeared uniform in size and were observed in large secretory vacuoles similar to those reported by Holtrop.[17] Small smooth vesicles, located between the GER and the Golgi complex, contained a moderately electron-dense material. These transition vesicles, approximately 110 nm in diameter, are assumed to transport products for packaging and secretion by the Golgi complex.

Proximal Region of the TD Lesion

At the ultrastructural level, there was no clear delineation between the lesion and the early hypertrophic zone. It contained chondrocytes with several different morphologic appearances (ie, necrotic cells, rarefied cells, condensing cells, and early hypertrophic cells), as shown in Figure 2.

Early Necrotic Cells

The early necrotic cells, 13–20 μ in diameter, were oval to spherical in shape, with numerous blebs and cytoplasmic projections (Figure 5). These chondrocytes did not contain a specialized cytoplasmic region with extensive Golgi and secretory vacuoles but, rather, the remnants of secretory vacuoles and distorted Golgi saccules, which were randomly distributed in the cytoplasm. The Golgi saccules were swollen (0.6–0.8 μ in diameter), compared with those in the early hypertrophic cell (0.4–0.5 μ). The secretory vacuoles were atrophic and undulated in shape and contained a fine fibrillar and granular material. Some vacuoles appeared to have a thin, uneven layer of moderately electron-dense material adhering to the inner surface of the membrane (Figure 6).

The cisternae of the GER were shorter and more swollen than early hypertrophic cells. They were filled with a moderately electron-dense material, like the early hypertrophic cell. The mitochondria, however, were swollen and distorted in shape. Cup-shaped and ring-shaped mitochondria were frequently observed in the early necrotic chondrocytes. Similar mitochondrial transformations have been seen in the liver after the administration of drugs which disturb metabolism, eg, carbon tetrachloride[19] and ammonium carbonate.[20]

The nucleus was similar in appearance to that of the early hypertrophic cell except for the presence of clusters of interchromatin granules, also called nuclear ribosomes. Interchromatin granules, which are abundant in tumor cells,[21] may represent early necrotic changes in the nucleus or altered nuclear activity.

Necrotic Cells

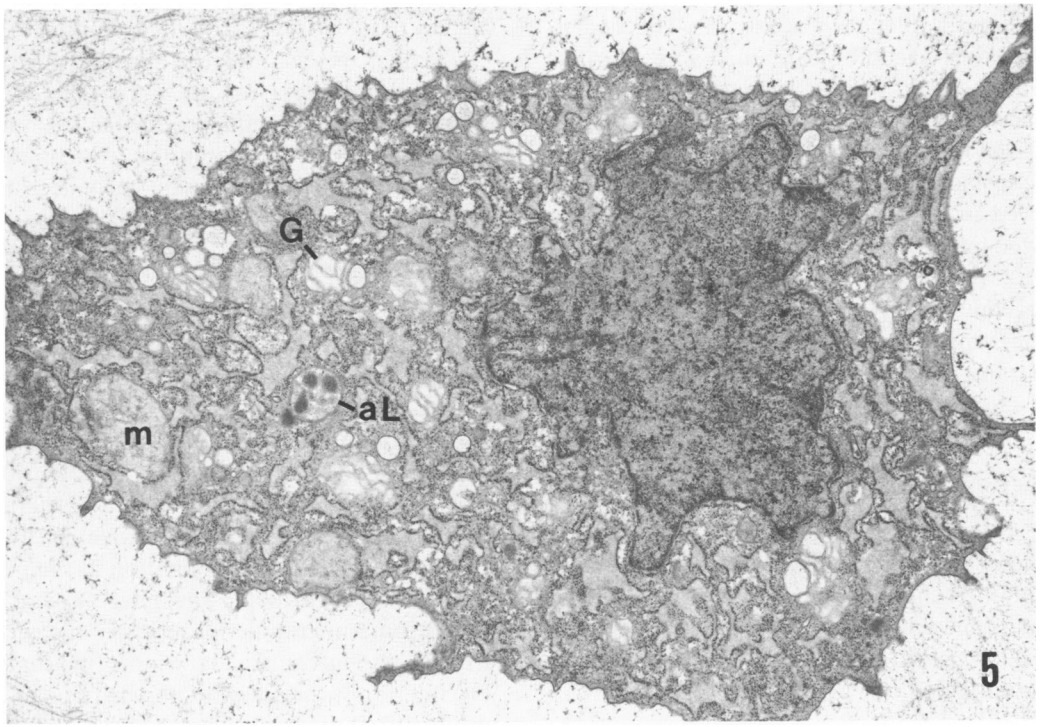
Deeper in the proximal lesion, cells with more advanced degenerative stages were observed. The most striking feature of the necrotic chondrocyte was the appearance of the nucleus and the GER (Figures 7 and 8). The contour of the cells was smoother, with smaller and fewer blebs and cytoplasmic extensions. These chondrocytes were approximately 11–15 μ in diameter, smaller than the early hypertrophic cell.

Nuclear chromatin margination at the periphery of the nucleus was evident. The nucleolus and clusters of interchromatin granules observed in the early necrotic cell may also marginate to the inner nuclear membrane. The interior of the nucleus consisted of a fine-textured, evenly distributed flocculent material. Small electron-dense aggregates of condensed chromatin were sometimes seen within the fine-textured material. The nucleus of the necrotic cell, unlike that of the early necrotic cell, did not have an undulating appearance. The paranuclear space was electron-lucent, ranged from 20 to 140 nm in width, and was smallest near the regions containing nuclear pores. Fine fibrillar material was seen adjacent to the nuclear membranes which bordered the paranuclear space.

The cytoplasm contained numerous vesiculated cisternae of the endoplasmic reticulum with diameters ranging from 18 to 535 nm. These vesicles appeared to be produced by the fragmentation of the GER. Partial degranulation of some vesicles was observed (Figure 8). The vesiculated cisternae were filled with a fine-textured flocculent material, similar to that found in the nucleus. Some vesicles contained an electron-dense material. These cytoplasmic structures contained clumps of a moderately electron-dense, flocculent material and were approximately 500–550 nm in diameter.

The plasma membrane of necrotic cells appears to be intact, because cellular debris was not observed in the territorial matrix. The mitochondria of necrotic chondrocytes were swollen and contained dilated in-

Figure 3—A typical chondrocyte from the early hypertrophic zone. *ER*, endoplasmic reticulum; *M*, rod-shaped mitochondria; *G*, Golgi saccules; *SV*, secretory vacuoles with fine particles of proteoglycans; *L*, lysosomelike bodies. Vacuoles appear ready to discharge their finely granular contents (*arrow*). (Uranyl acetate and lead citrate, × 13,300) **Figure 4**—Higher magnification of an early hypertrophic chondrocyte. A secretory vacuole appears to be continuous with the Golgi saccules (*arrow*). Transition vesicles (*T*) are observed between the GER and the forming face of the Golgi complex. (Uranyl acetate and lead citrate, × 46,000)



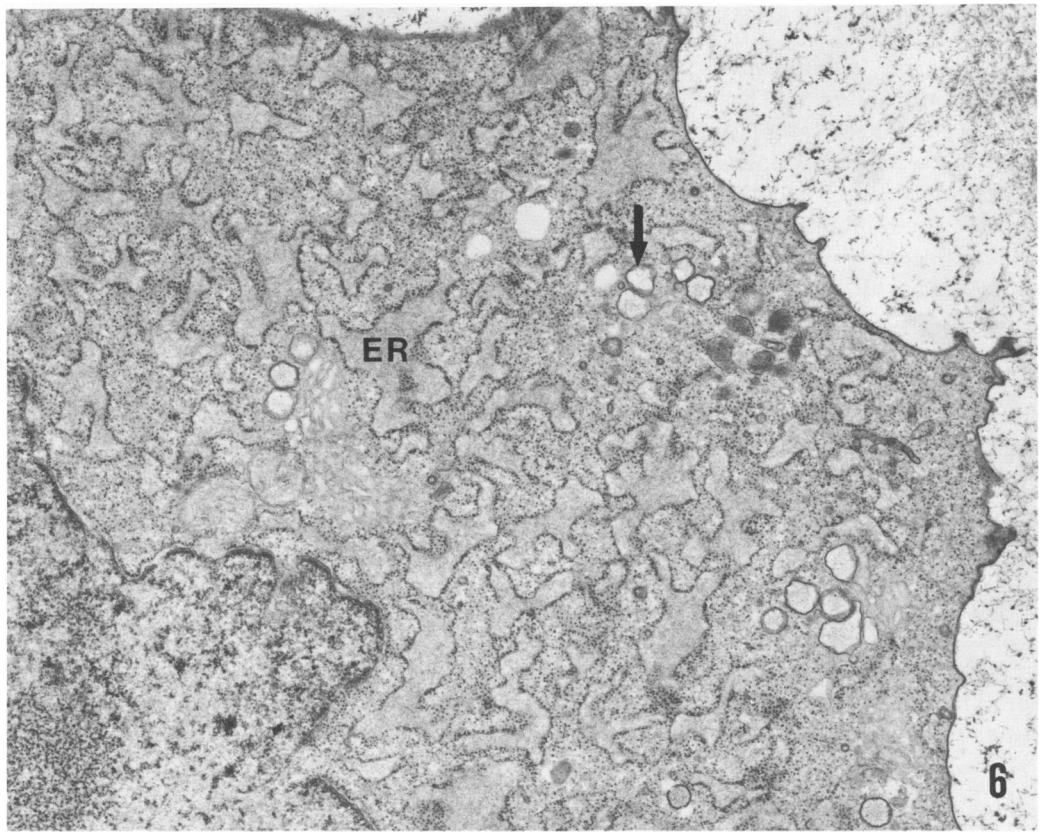
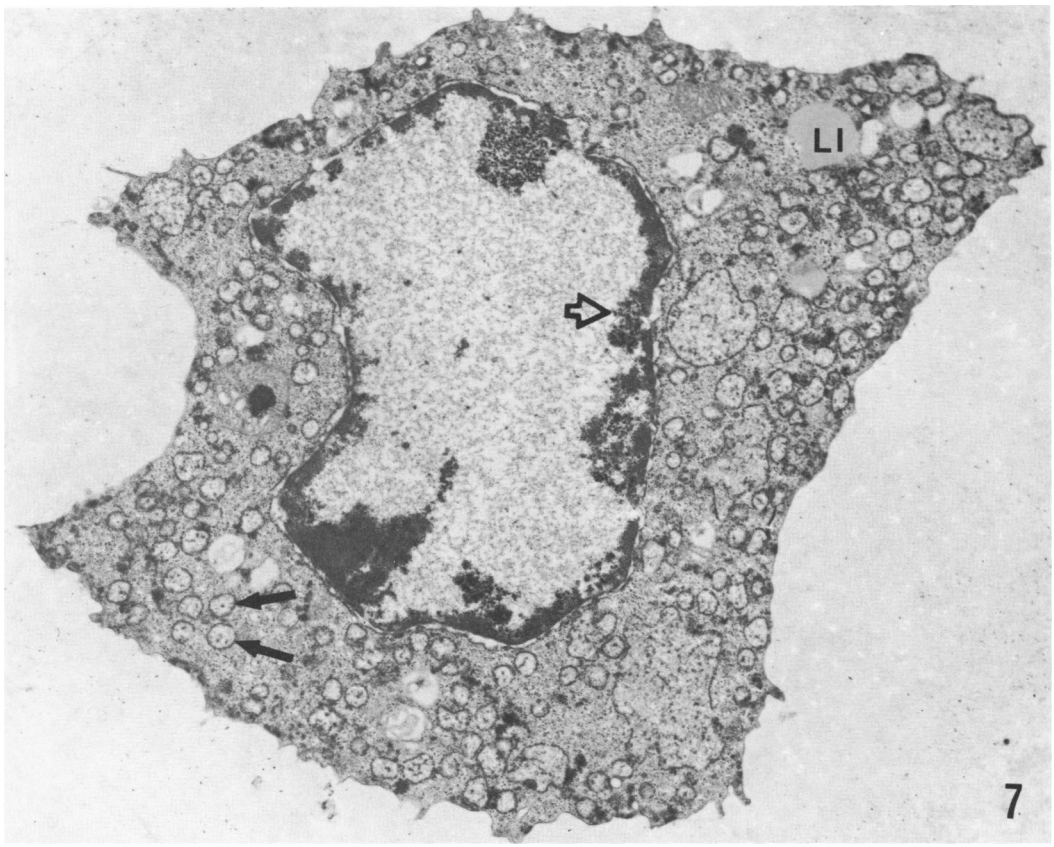


Figure 5—Early necrotic cell from the proximal region of the dyschondroplastic lesion. *M*, swollen mitochondrion; *G*, dilated Golgi saccules; *aL*, autolysosome. (Uranyl acetate and lead citrate, ×9500) **Figure 6**—Early necrotic cell at a higher magnification. Exhibited are dilated cisternae of the endoplasmic reticulum (*ER*) and atrophic secretory vacuoles (*arrow*) which are smaller than normal in size. (Uranyl acetate and lead citrate, ×15,000)



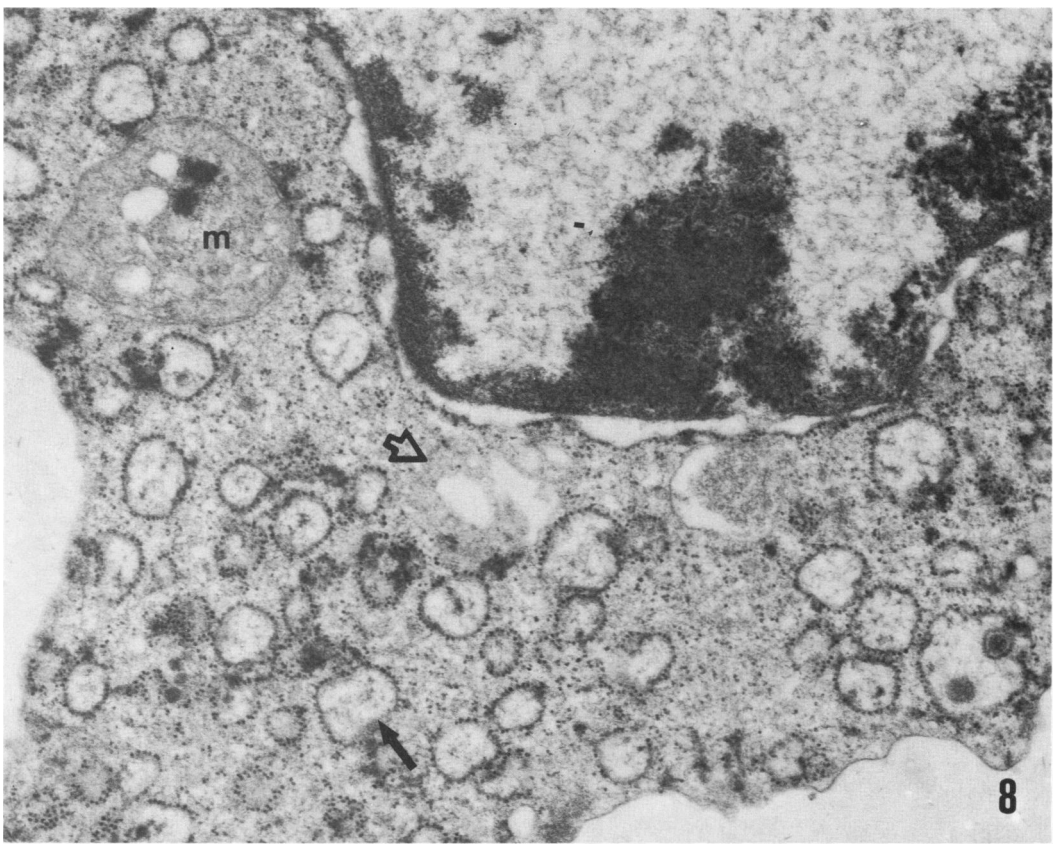
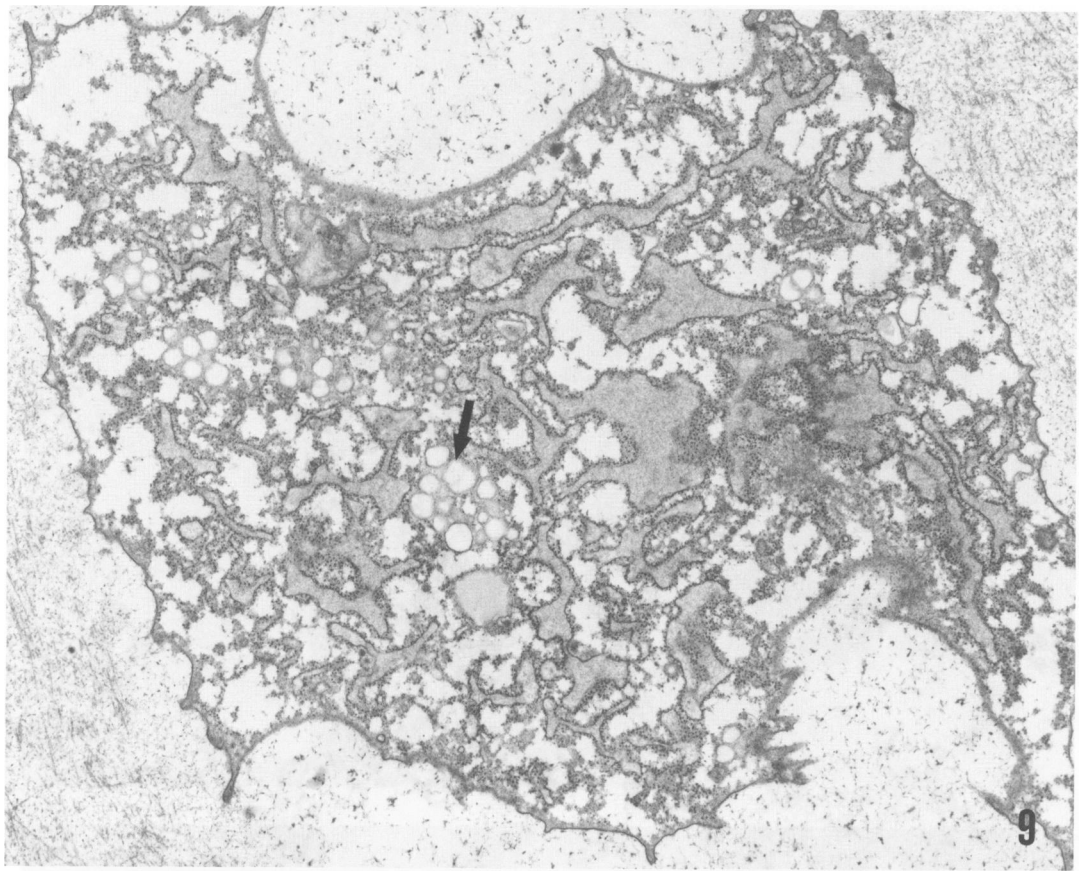


Figure 7—Necrotic cell from the proximal region of the lesion. Characteristic features are marginated chromatin (*open arrow*), lipid inclusions (*LI*), vesiculated cisternae of the endoplasmic reticulum (*solid arrows*), and a paranuclear space. (Uranyl acetate and lead citrate, ×9500) **Figure 8**—Necrotic cell at higher magnification. Of particular note are the degranulated regions of the vesiculated endoplasmic reticulum (*arrow*) and swollen mitochondrion (*M*). Remnants of the Golgi saccules (*open arrow*) are present in the cytoplasm. (Uranyl acetate and lead citrate, ×27,500)



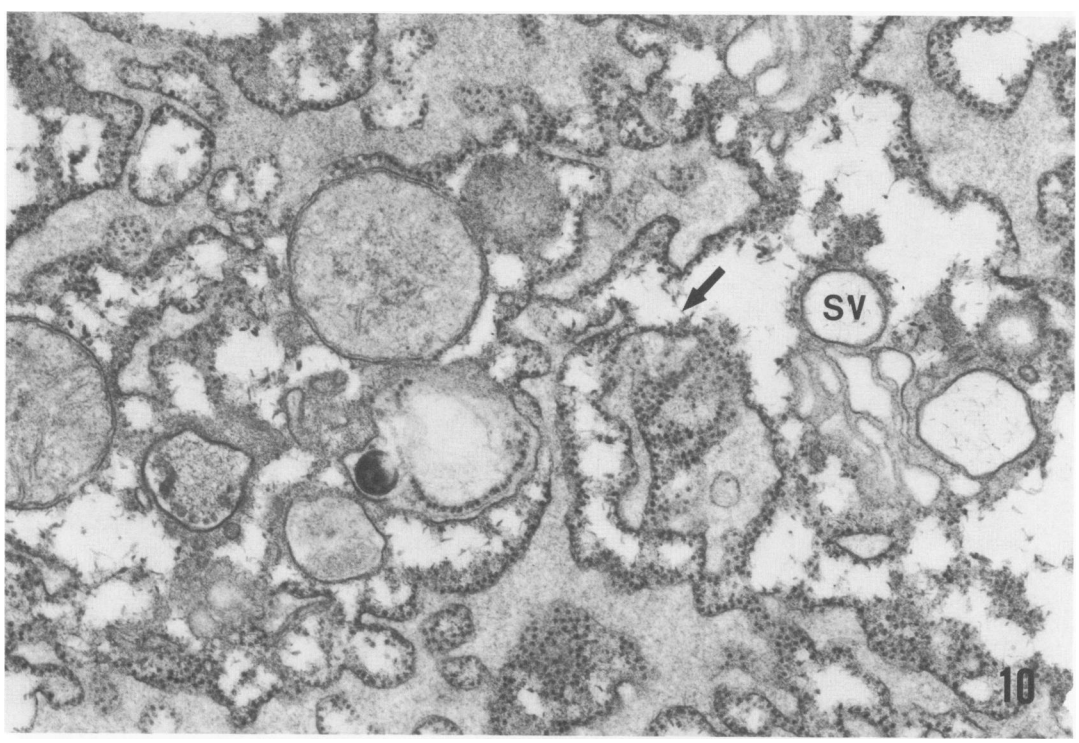


Figure 9—Rarefied cells from the proximal region of the lesion. Typically these cells exhibit swollen Golgi saccules (*solid arrow*), lipid inclusions, and ribbons of endoplasmic reticulum with some dilated cisternae. The organelles are dispersed and separated from each other. (Uranyl acetate and lead citrate, × 13,300) **Figure 10**—Rarefied cells at higher magnification showing clumps of ribosomes and filamentous cytoplasmic material adjacent to the dilated endoplasmic reticulum. The filamentous material (*arrow*) may represent elements of the microtrabecular lattice. The mitochondria appear similar to those observed in Figures 5 and 6. The secretory vacuoles (*SV*) are atrophic and small in size. (Uranyl acetate and lead citrate, × 34,700)

tracristal spaces, approximately 140 nm in diameter. Electron-dense, flocculent material occurred in the intramitochondrial matrix.

Rarefied Cells

Other chondrocytes that were observed in the proximal region of the dyschondroplastic lesion were enlarged and rarefied. These occurred at about one-third the frequency of nonrarefied necrotic cells. The rarefied chondrocytes were usually spherical but sometimes assumed other shapes (Figures 9 and 10). The cytoplasmic features of these chondrocytes, except for the swollen and hydrated appearance, resembled the early necrotic cells.

Condensing Cells

A few chondrocytes in the proximal region of the TD lesion were condensed, forming a dense osmiophilic mass (Figure 2). Condensing chondrocytes are polymorphic because there are several cell types which condense: rarefied cells, necrotic cells, and early necrotic cells. There are some structures of condensing cells which are common, and may, in fact, function in the process of condensation or dehydration. In many chondrocytes, the cisternae of the endoplasmic reticulum appeared larger, whereas the intracytoplasmic matrix was condensed (Figure 11). The membrane of the endoplasmic reticulum appeared to fuse with the plasma membrane (Figure 12), a possible site of release of cellular fluid.[22] The dilated endoplasmic reticulum formed an interconnecting network which enclosed groups of organelles and membranous structures, forming entrapped islands of cytoplasmic organelles. These cytoplasmic structures may then fuse with lysosomes, forming autophagic vacuoles, which are observed in the condensed necrotic cell. The membranous structures observed in the entrapped islands or lamellar bodies may represent remnants of secretory vacuoles and Golgi complex.

Condensing necrotic cells have different sizes, depending upon the amount of cellular fluid. These chondrocytes may contain blebs, but the slender cytoplasmic extensions, observed in the early hypertrophic cell, were not common.

Apoptosis

Apoptotic chondrocytes, cells undergoing physiologic cell death,[22] were occasionally observed in the proximal and mid regions of slightly dyschondroplastic lesions. These cells contained the characteristic nuclear appearance, indicative of apoptosis (Figure 12). Apoptotic chondrocytes were not observed in severe TD lesions. The apoptotic cell in Figure 12 has become necrotic, which is evident by the appearance of the mitochondria.

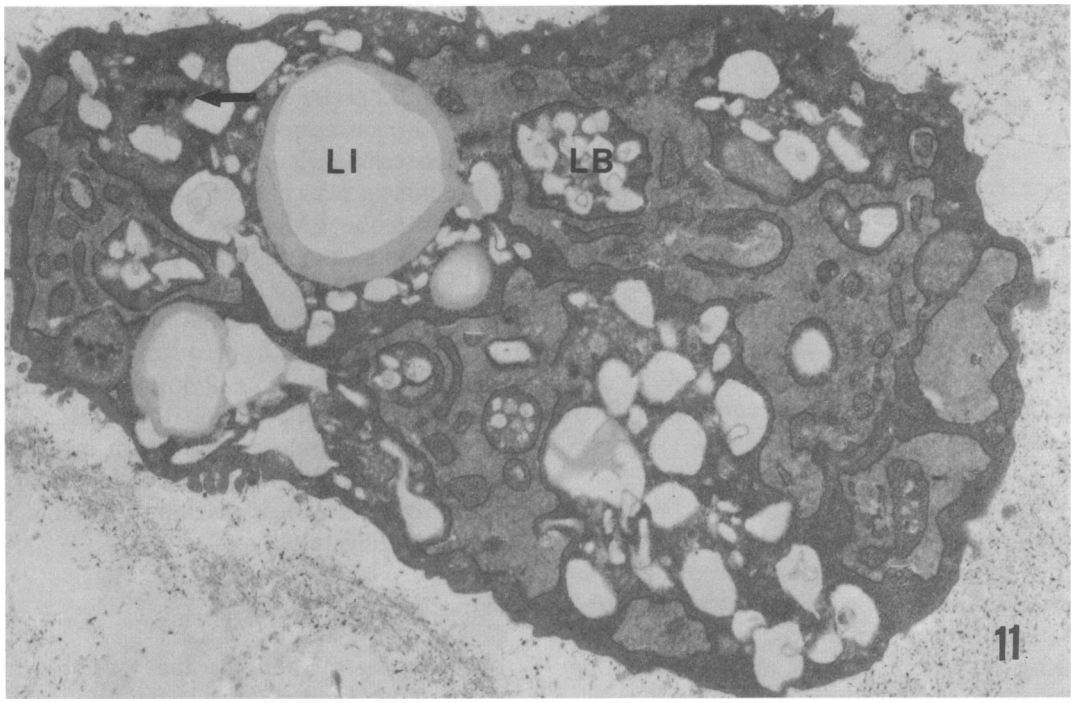
Mid and Distal Regions of the TD Lesion

The chondrocytes of the mid and distal regions of the severe TD lesion were condensed osmiophilic masses, measuring approximately 2–9 μ in size (Figure 13). These cells appeared convoluted and distorted, with intracellular spaces containing fine fibrillar material, which is probably internalized proteoglycan. Some cytoplasmic structures, termed autophagic vacuoles, resembled the lamellar bodies observed in the condensing necrotic cells. The contour of the condensed autolytic cell is not smooth, because of the discontinuity of the plasma membrane. Pyknotic and karyorrhexic nuclei are commonly found in these autolytic masses. The clumping of nuclear chromatin, karyorrhexis, is shown in Figure 13. The condensed chromatin may also retract from the nuclear envelope, forming a pyknotic nucleus (Figure 14).

Matrix and Cellular Debris

The matrix surrounding the chondrocytes in all regions of the lesion contained a fine fibrillar and granular material which was normal in appearance (Figures 3, 5, 7, 9, 11–13, and 15). Various types of cellular debris were observed in the territorial matrix of condensed necrotic cells (Figure 15a). Aggregates of thick collagen fibers, termed microscars, were seen in the vicinity of degenerating chondrocytes, similar to those described by Silberberg.[15] These focal aggregates varied from 0.30 to 0.65 μ in size. They are not associated with any specific abnormality but have been observed in both normal and pathologic conditions. Osmiophilic globules, approximately 105 nm in diameter, were also observed near degenerating cells. These amorphous structures appeared to derive from the electron-dense osmiophilic masses of deteriorating chondrocytes. Matrix vesicle-like structures approximately 0.15 μ in diameter were also observed in the territorial matrix of degenerating chondrocytes in the distal region. The territorial matrix is an abnormal location for matrix vesicles. Membranous structures represented the usual form of cellular debris (Figure 15b) and were found in close proximity to the condensed autolytic cells in the mid and distal regions of the TD lesion. These membranous bodies, which were approximately 60 nm thick, consisted of cellular membranes with proteoglycan granules and small segments of collagen fibers.

The interterritorial matrix contained matrix vesicles, proteoglycan granules, collagen fibrils, and fine filaments (Figure 16). Matrix vesicles of approximately 0.1 μ in diameter were observed at all levels of the lesion. Proteoglycan granules, which were pleomorphic in shape, were approximately 28 nm in diameter. Fine filaments, extending from the matrix granules (ie, pro-



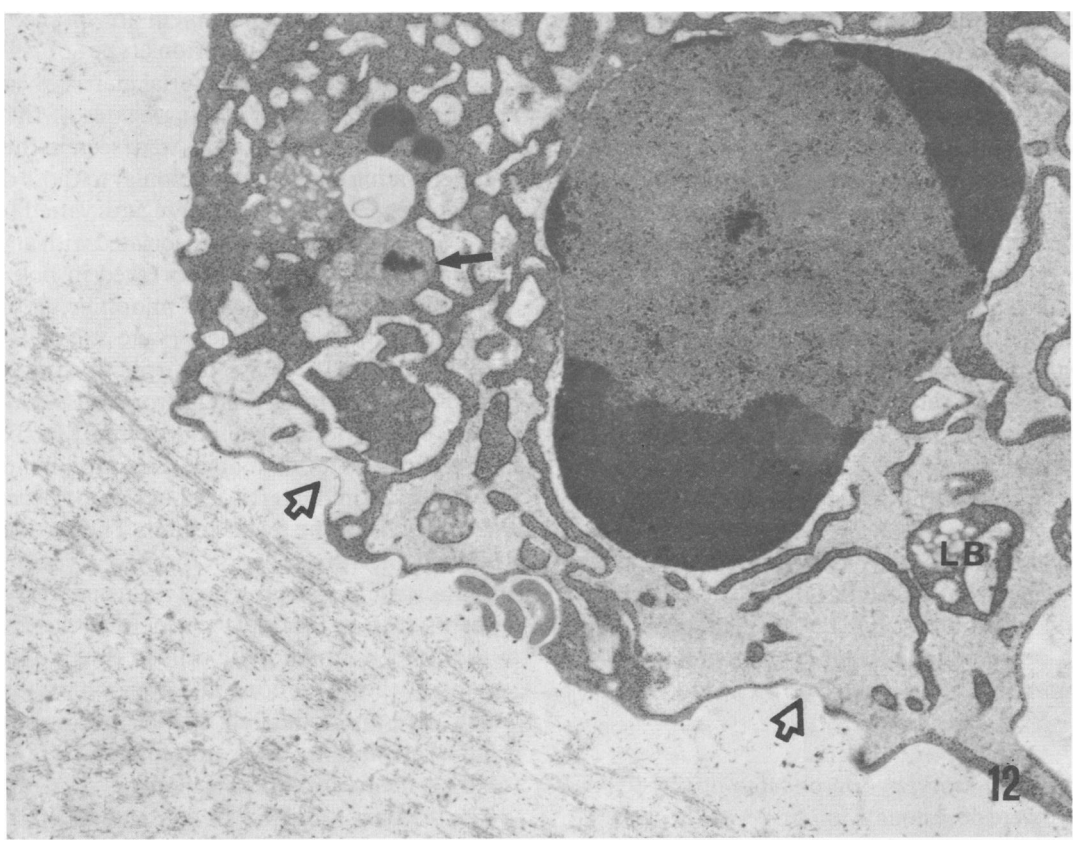
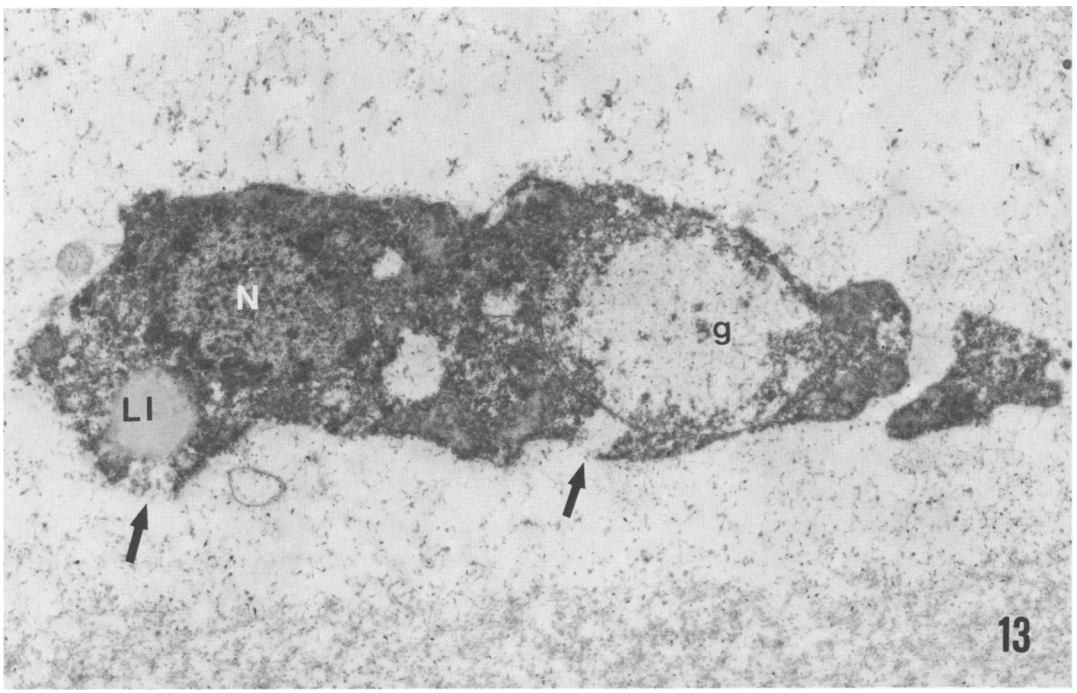


Figure 11—Condensing necrotic chondrocyte from the proximal region of the lesion. The dilated cisternae of the endoplasmic reticulum have enlarged and formed a continuous network. The number of cytoplasmic projections has significantly decreased, compared with that of the early hypertrophic chondrocyte. The cytoplasm is dense, possibly because of a loss of water. *LI*, lipid inclusion; *LB*, lamellar body; *arrow*, mitochondrion. (Uranyl acetate and lead citrate, × 14,000) **Figure 12**—Apoptotic chondrocyte from the proximal region of the lesion. This type of cell is characterized by the appearance of the nucleus, which contains outwardly bulging crescentic caps of condensed chromatin, lamellar bodies (*LB*), swollen endoplasmic reticulum, and often has degenerating mitochondria (*solid arrow*). The fusion of the endoplasmic reticulum and plasma membrane (*open arrows*) may facilitate the removal of cellular fluid. (Uranyl acetate and lead citrate, × 13,300)



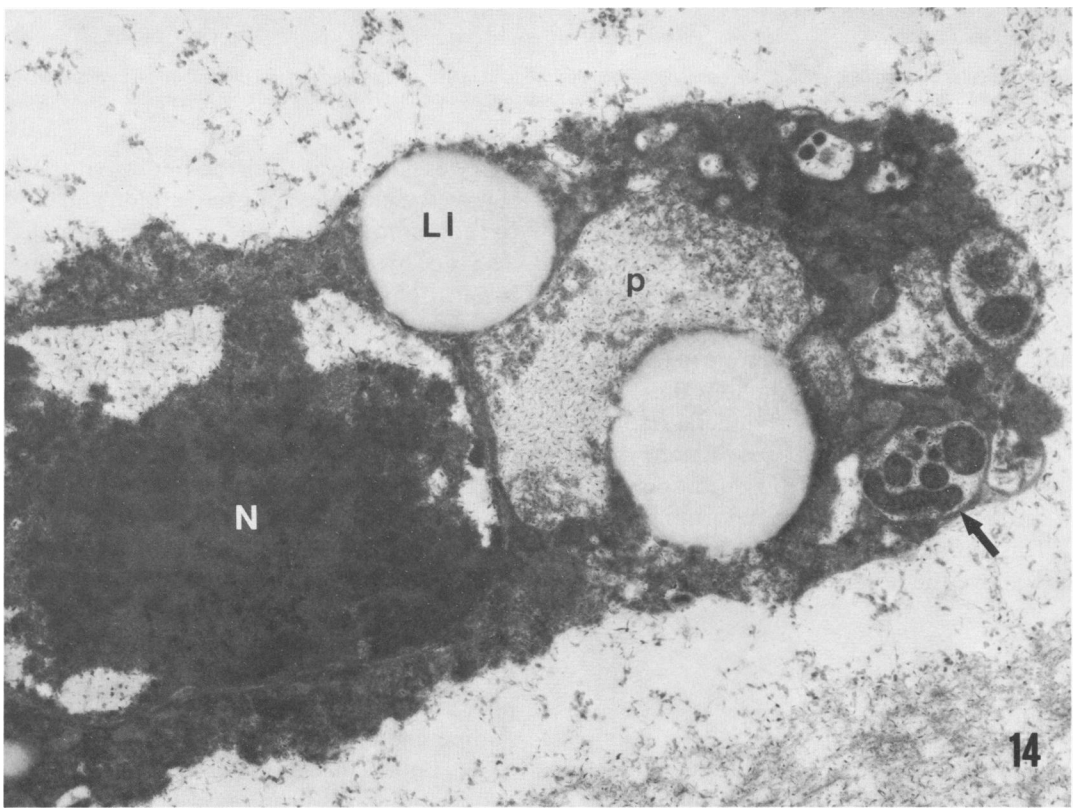


Figure 13—Condensed necrotic cell from the distal region of the lesion. *N*, karyorrhexic nucleus; *LI*, lipid inclusions; *g*, internalized granular material; *arrows*, discontinuity of the plasma membrane. (Uranyl acetate and lead citrate, ×16,200) **Figure 14**—Condensed necrotic cell at a higher magnification showing autolysosomes (*arrow*), lipid inclusions (*LI*), internalized proteoglycan granules (*p*), and a pyknotic nucelus (*N*). (Uranyl acetate and lead citrate, ×32,000)

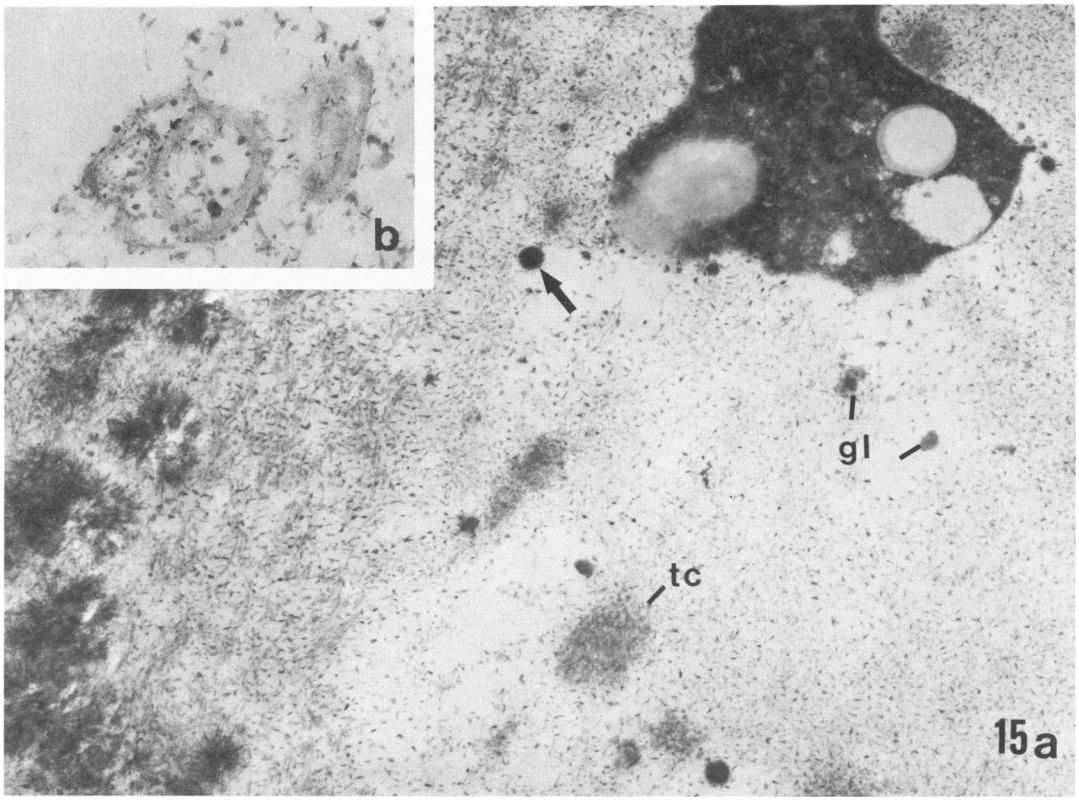


Figure 15a—Aggregates of thick collagen fibers (*tc*), osmiophilic globules (*gl*), and matrix vesicles (*arrow*) are present in the territorial matrix of a condensed necrotic cell. (× 18,800) **b**—Membranous structures were the most frequently observed type of cell debris in the territorial matrix. (× 32,600)

teoglycan granules), may interconnect with other granules and collagen fibrils. Unaggregated collagen fibrils were approximately 17–28 nm thick.

Most of the matrix vesicles in the distal region of the dyschondroplastic lesion were calcified and were approximately 180–225 nm in size (Figure 17). The mineralized nodules, presumed to be derived from matrix vesicles, appeared to enlarge to approximately 365 nm in size and then coalesce to form large mineral nodules. Cells also calcify at the distal edge (see Figure 7 in the third paper in this series).[23] Mineralization occurred abruptly at the distal edge of the lesion, forming a shell of mineral. This was the sole site of mineralization. The growth plate above the lesion did not calcify.

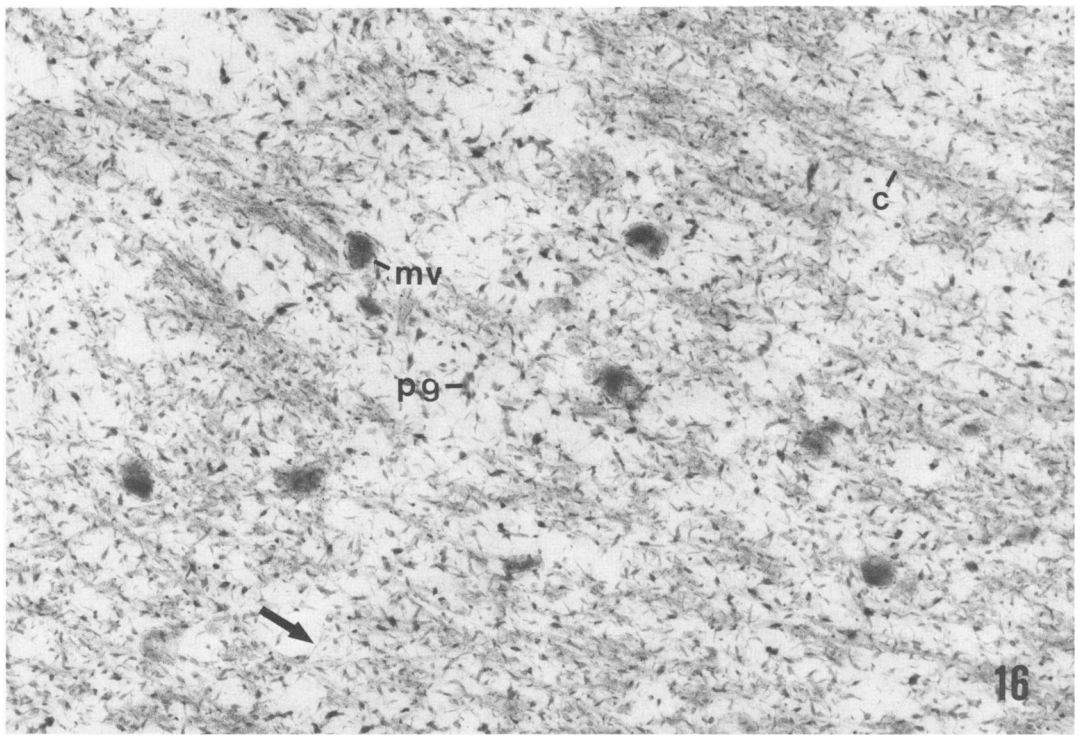
Discussion

Chondrocytes of the Lesion

The ultrastructural analysis of the TD lesion and early hypertrophic zone of the growth plate adjacent to the lesion has revealed that the cells have entered a progressively worsening condition and eventually die. In agreement with the findings of Poulos,[5] we found that the chondrocytes do not completely mature or hypertrophy. In the normal growth plate, as the chondrocytes move away from the epiphyseal arteries, which supply the nutrients for proliferation and development of the chondrocytes,[24] the oxygen tension decreases to a level of hypoxia.[25] Brighton[26] attributes chondrocyte hypertrophy to hypoxic conditions and limited anaerobic metabolism. The release of mitochondrial granules and the formation of matrix vesicles may be a response of the cell to the hypoxic environment.[27] Kardos and Hubbard[28] suggest that sublethal damage to the chondrocytes results in the budding of matrix vesicles.

Poulos[5] characterized the blood vessel supply in turkey dyschondroplasia by angiography. He showed that the epiphyseal arteries which feed the developing growth plate and which extend to the prehypertrophic zone were normal. The metaphyseal vessels which approach the lesion both distally and laterally only penetrated into the lesion about 1 μ, then turned back. The blood supply to the lesion appeared to be insufficient for maintaining chondrocyte metabolism.

The importance of the metaphyseal blood vessels in endochondral ossification was demonstrated by Trueta and Amato.[24] They performed surgical ablation of the metaphyseal blood vessels and observed the formation of a metaphyseal cartilaginous lesion in the proximal tibia of rabbits. In a similar experiment, Riddell[29] was able to produce a cartilaginous lesion in the metaphy-



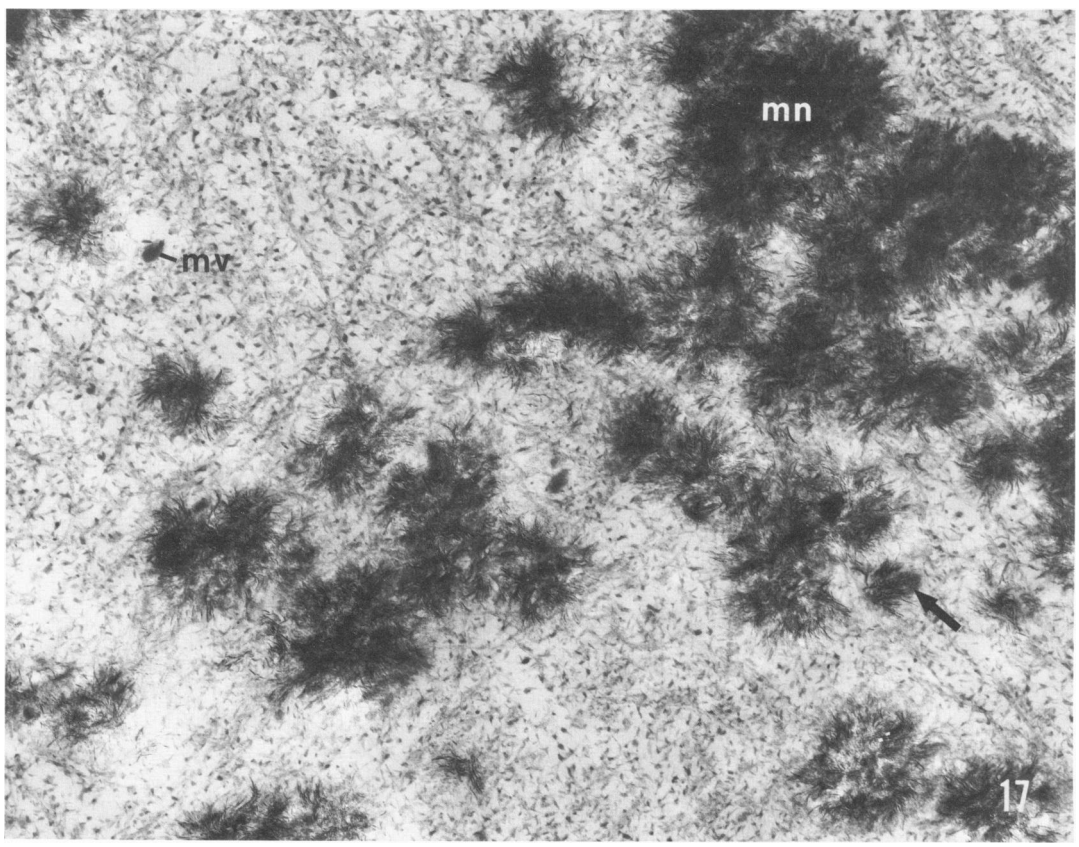


Figure 16—The extracellular matrix from the proximal region of the lesion. This normal-appearing matrix contains matrix vesicles (*mv*), proteoglycan granules (*pg*), and collagenous fibrils (*c*). The fine filaments (*arrow*) seem to connect proteoglycan granules to the collagen fibrils and other matrix granules. (Uranyl acetate and lead citrate, ×35,200) **Figure 17**—The extracellular matrix from the distal portion of the lesion. This matrix contains matrix vesicles (*mv*), small mineral nodules which appear to have arisen from matrix vesicles (*arrow*), and large mineral nodules (*mn*). (Uranyl acetate and lead citrate, ×27,500)

sis of the proximal tibiotarsus, which appeared similar to tibial dyschondroplasia. TD, therefore, may result from the blockage of vascular tunneling of the metaphyseal blood vessels into a lesion, a condition mimicking vascular ablation. A reduced vascular supply from the metaphyseal vessels could create an anoxic environment or one in which the O_2 levels are extremely low as the growth plate lesion thickens. The ultrastructural changes which occurred in TD chondrocytes have also been demonstrated in other cell types in culture under anoxic conditions[30] and *in vivo* with the use of metabolic inhibitors.[31] Enlargement of the intracristal spaces and amplitude swelling of mitochondria, observed in early necrotic cells, is indicative of extremely low O_2 and ATP. Low O_2 levels are known to reduce mitochondrial phosphorylation.[32] The resulting decreased ATP levels stimulate phosphofructokinase, and this results in an increased rate of anaerobic glycolysis. Specific latent isozymes of lactate dehydrogenase are induced[33] which convert pyruvate to lactate. Increased acidity in the chondrocytes produced by anaerobic glycolysis would probably play a role in the death of the cells.

The findings of this study are in agreement with the work of Lilburn and Leach,[13] who showed that TD chondrocytes have a very low rate of oxidative metabolism. It is thought that, in the absence of ATP, protein synthesis is inhibited and is manifested by the vesiculation of the rough endoplasmic reticulum.[30] The appearance of electron-dense, flocculent material in the mitochondria, representing accumulation of fatty acids and denatured protein,[34] indicates that endogenous phospholipases may not have been activated by ionized calcium.[35] Flocculence in mitochondria is an indication of irreversible cell death.[30]

Autolysis of the necrotic chondrocytes ultimately produces autolytic masses in the mid and distal TD regions. Lowther et al[34] demonstrated that a decreased biosynthetic activity of approximately 92% occurred in the lesion. These intracellular changes appeared to stem from an anoxic milieu. The chondrocytes, being necrotic and autolytic, appear to be unable to alter the extracellular matrix to stimulate angiogenesis of the metaphyseal vessels.

The presence of lipid inclusions in necrotic cells implies that the lipids were resistant to cellular autolytic enzymes. The electron-lucent appearance of the lipid inclusions implies that they are rich in saturated fatty acids, since osmium tetroxide binds mainly to unsaturated fatty acids. The annular appearance of some of the droplets is probably due to incomplete fixation.

Extracellular Matrix

Poulos[5] suggested that the failure of vascular penetration was due to the extracellular matrix. His suggestion is supported by some earlier histochemical work. Siller[12] demonstrated that the dyschondroplastic cartilage did not show positive staining for toluidine blue, alcian blue, or Hale's colloidal iron. PAS staining clearly distinguished between normal and dyschondroplastic tissue.[1] Lowther et al[36] however, showed biochemically that the composition and content of proteoglycans are nearly identical in the normal proliferative zone and the TD lesion. This has been corroborated by Freedman et al.[37]

Ultrastructurally, the interterritorial and territorial matrixes of the dyschondroplastic lesion contain all the structural elements, such as matrix granules and collagen fibrils, of the normal epiphyseal plate. These components of the TD extracellular matrix appeared identical to those of the normal epiphyseal growth plate, except that the collagen fibrils were on the thin side, having a thickness of 17–28 nm. Type II collagen, which occurs in normal cartilage, has a thickness of 25–50 nm.[38]

Similarities to Other Skeletal Dysplasias

Other types of skeletal dysplasias which occur in humans contain condensed degenerating chondrocytes. Diastrophic dwarfism is a generalized abnormality of the resting cartilage which secondarily affects the epiphyseal plate. Ultrastructural studies of this disease in the costochondral junction have demonstrated degenerating chondrocytes with large lipid inclusions, surrounded by heavy collagen fibrils.[39] Another abnormality, Dyggve-Melchior-Clausen syndrome, is characterized by short-trunk dwarfism. In this disease, the resting cartilage, like TD, contains dead cells, either whole or in the form of cellular debris. The condensed autolytic chondrocytes are surrounded by aggregates of thick collagen fibrils, osmiophilic globules, and calcium deposits.[15] In the most common type of chondrodystrophy, achondroplasia, the iliac crest contains an unusually large number of dead cells.[15] Achondroplasia is characterized by short-limbed, short stature and is produced genetically by an autosomal dominant trait.

Metaphyseal chondroplasias, like TD, contain tonguelike extensions of cartilage into the metaphysis of bone. The chondrocytes, however, are large in appearance and are arranged in clusters, surrounded by dense collagen fibrils.[39] The ultrastructure of the cells is characterized by dilated rough endoplasmic reticulum similar to that observed in the early necrotic cells of the TD lesion.[40]

Summary and Conclusions

The earliest sign of the dyschondroplastic lesion that was detectable by electron microscopy was the appear-

ance of necrotic cells adjacent to the early hypertrophic cells of the growth plate. Full hypertrophy, calcification, and vascularization[36] of the growth plate did not occur. In the proximal region of the lesion, chondrocytes began to show signs of condensation. The mid region, which occupies the bulk of the lesion, was distinguished by masses of condensed osmiophilic bodies, representing dense autolytic aggregation of denatured protoplasm. The distal region of the TD lesion is hallmarked by extensive condensation of the cells and by the calcification of matrix vesicles in the interterritorial matrix, forming mineral nodules which coalesced to form a shell of mineral at the distal edge of the lesion. TD chondrocytes exhibited ultrastructural changes indicative of an energy depletion such as mitochondrial swelling and the accumulation of electron-dense, flocculent material in the matrix. This implies that the availability of oxygen and other nutrients within the lesion is inadequate. The necrotic changes progressively worsened in chondrocytes more distal to the epiphyseal arteries. This study supports the view, then, that hypoxia produces hypertrophy and anoxia causes necrosis. Autolysis of the necrotic cells produced condensed osmiophilic masses with large lipid inclusions which were not affected by autolysis. In the extracellular matrix, the structural components, such as collagen fibrils and matrix vesicles, appeared normal, compared with those of the normal epiphyseal plate. However, in the mid and distal regions matrix vesicles and various types of cellular debris were observed in the territorial matrix adjacent to the autolytic masses. Apoptotic cells were occasionally observed in the proximal region of the slight lesion, but not in the severe lesions. This indicates that chondrocytes, under favorable conditions, have the capacity to undergo normal physiologic death. This also supports the view of Kardos and Hubbard[28] that matrix vesicle generation represents a response of chondrocytes to sublethal injury, possibly the result of hypoxia. The fact that matrix vesicles did not calcify in mid lesion, but only at the distal edge of the lesion, suggests that diffusion of nutrients such as calcium and phosphate into the lesion is inadequate. Determination of the elemental composition in ashed lesions[37] and in thin sections by electron microprobe analysis[23] also indicates that this is the case. The shell of mineralization at the distal edge of the lesion was formed by the calcification of both cells and matrix.

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